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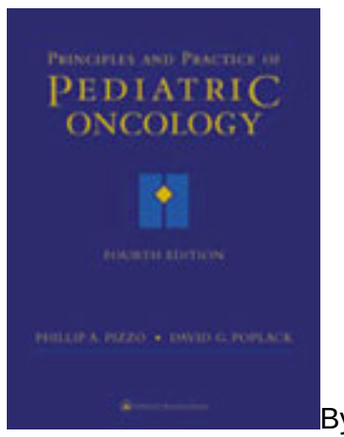

By OkDoKeY

# Principles & Practice of Pediatric Oncology

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# PREFACE

The publication of the fourth edition of *Principle and Practice of Pediatric Oncology* is occurring at one of the most exciting times in medical history. With the recent completion of the human genome project and the impressive advances in molecular and cellular biology, medical scientists now have at hand extraordinary information and technologies that promise to revolutionize our understanding of human diseases and dramatically improve our ability to diagnose, treat, and prevent them. Although its ultimate benefits are impossible to accurately predict, there is little doubt that the field of genomics holds extraordinary promise for the study of childhood malignancies.

In 1989, when the first edition of *Principles and Practice of Pediatric Oncology* was published, remarkable improvements in the treatment of childhood cancer had already occurred, but the ability to identify genes associated with specific malignancies was nascent. Now, advanced genomic technologies make it likely that, within the next 5 to 10 years, we will identify those genes unique to pediatric malignancies that are responsible for their development, progression, metastases, and resistance to therapy. Equally important, the comprehensive application of functional genomics, proteomics, and molecular pharmacology is likely to lead to the identification of more specific, molecularly targeted therapeutic approaches. In addition, application of these technologies to areas such as molecular epidemiology eventually may make prevention of pediatric cancer a reality. These technologic advances pose a significant challenge to the modern day pediatric oncologist who, in addition to possessing a vast knowledge of traditional clinical pediatric oncology, must have a thorough understanding of the “new biology” and its relevance to pediatric malignancies and their treatment.

To help meet these challenges, the fourth edition of *Principles and Practice of Pediatric Oncology* offers expanded discussions of the molecular biology and genetics of pediatric cancers. Where possible, emphasis has been placed on those recent findings that may provide the basis for the development of innovative therapeutic strategies in the future.

At the same time, the fourth edition of *Principles and Practice of Pediatric Oncology* continues to offer the equally important comprehensive reviews of the diagnosis and treatment of pediatric malignancies. In addition, the fourth edition provides in-depth discussion of the fundamental principles of clinical management and supportive care. The critical importance of offering strong psychosocial support for patient and family is stressed, as is the necessity of embracing a multidisciplinary approach to care for these patients. As with past editions, this new edition offers guidance on how physicians should collaborate with members of the multidisciplinary team to ensure that each child has the best options for living and, when cure is not possible, that death occurs with as much dignity as possible for both the child and family.

We have attempted to produce a book that is an essential resource to all those involved in the care of children with cancer. We are indebted to our many colleagues, each an expert in his or her field, who, by serving as authors, have contributed their knowledge to this endeavor. We are grateful for their time, energy, and wisdom. We also would like to thank Sharon Olsen, who served as our editorial assistant, and Mariann Waldbillig for their extraordinary hard work and loyalty to this project. Their contributions are invaluable. We want to thank Jonathan W. Pine, Jr., Acquisitions Editor, and Tanya Lazar, Developmental Editor, from Lippincott Williams & Wilkins and Sophia Elaine Battaglia, Production Editor, from Silverchair Science + Communications for their guidance and assistance in the preparation of this edition. Through each of the past editions to the current edition, our commitment to the contiguity and excellence of *Principles and Practice of Pediatric Oncology* has been consistent and unwavering for our authors, readers, and the children and families we serve.

*Philip A. Pizzo, M.D.*
*David G. Poplack, M.D.*

## COLOR FIGURE SECTION

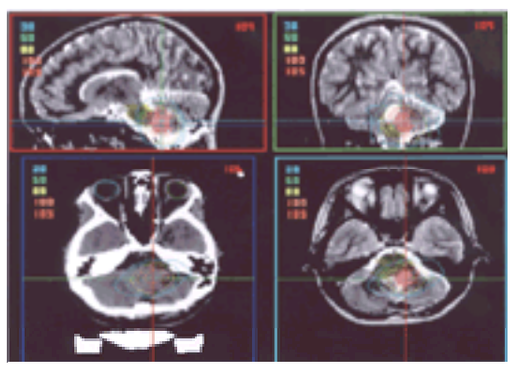

**FIGURE 13-2.** The patient is a 12-year-old girl with a low-grade astrocytoma. The dose distribution is shown superimposed on the sagittal, coronal, and transverse magnetic resonance (MR) sections through the center of the radiation field (radiation isocenter). The corresponding transverse computed tomography (CT) slice is also shown (*lower left*) in contrast to the soft tissue contrast of MR (*lower right*). Radiation treatment planning relies on both CT and MR modalities. The CT is required to accurately calculate the radiation inside the patient, whereas the MR in this case is required to reconstruct the target volume and the nearby brainstem. Dose lines are shown in percent of dose prescription of 50.4 Gy delivered in 25 fractions of 1.8 Gy. (See black and white Figure 13-2.).

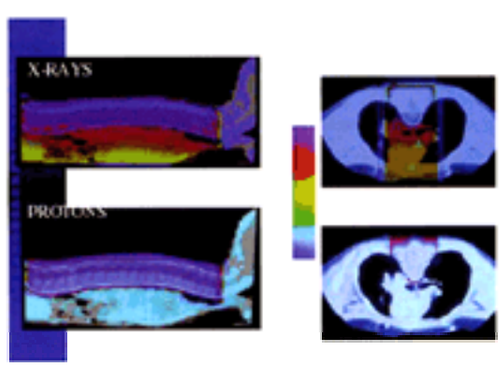


**FIGURE 13-10.** Shown here is the radiation dose in a 43-month-old child with medulloblastoma. The top figures demonstrate the dose from conventional x-rays given to the spine. Because x-rays deposit their energy over a greater distance, 60% of the dose exits to the stomach and intestine. The bottom figures are for protons showing no exit dose to the gastrointestinal tract, lung, or heart. (See black and white Figure 13-10.).

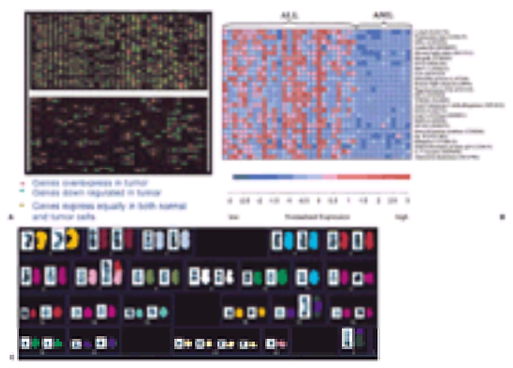

**FIGURE 19-6.** Application of new techniques for the molecular and cytogenetic characterization of acute lymphoblastic leukemia (ALL) blasts. **A:** Complementary DNA/messenger RNA (cDNA/mRNA) microarray. There are several current versions of this technology, including spotted cDNA arrays and oligonucleotide arrays. This example is of a cDNA array undergoing competitive hybridization from two sources of fluorescent-labeled RNA. The relative expression of each of 6,000 genes is measured by quantifying the amount of light emitted at the designated wavelengths at each spot on the array. **B:** Clustering of expression data on 11,000 genes (on an Affymetrix oligonucleotide array) from 38 cases of leukemia, showing that it is possible to use this technology to see differences as well as similarities among disease cases. (From Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science 1999;286:531–537, with permission.) **C:** Spectral karyotyping (SKY) on leukemic ALL blast cells. This case is remarkable for aneusomy (abnormal number) of chromosomes 21 and 22 as well as several complex marker chromosomes [45, der(X)t(X;17)(p21;q24), –Y, der(7)t(5;7)(a12;p22), and ider(17)(q10)t(X;17)(?q11)]. AML, acute myelogenous leukemia; IL-7, interleukin-7. (SKY results courtesy of X.Y. Lu, C.P. Harris, C.C. Lau, and P.H. Rao, *personal communication*, 2001.) (See black and white Figure 19-6.).

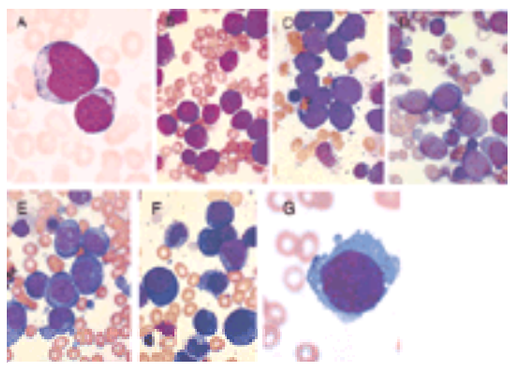

**FIGURE 20-2.** Morphologic French-American-British subtypes of acute myelogenous leukemia: **(A)** M1, **(B)** M2, **(C)** M3, **(D)** M4, **(E)** M5, **(F)** M6, **(G)** M7. May-Grunwald staining was used in all frames. (See black and white Figure 20-2.).

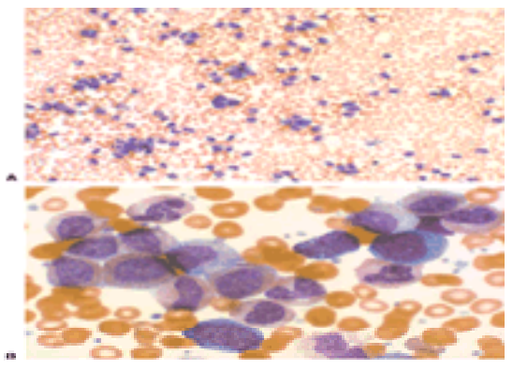

**FIGURE 21-6.** Peripheral blood smear of chronic-phase chronic myelocytic leukemia. **A:** Low-power magnification showing marked leukocytosis. **B:** High-power magnification showing the entire range of myeloid cells from myeloblast to mature polymorphonuclear leukocytes. (Courtesy of Dr. William Rezuke.) (See black and white Figure 21-6.).

# 1

# CHILDHOOD CANCER: INCIDENCE, SURVIVAL, AND MORTALITY

MALCOLM A. SMITH
LYNN A. GLOECKLER RIES



## INTRODUCTION

Cancer among children is relatively uncommon, with approximately 1 in 7,000 children 0 to 14 years of age being newly diagnosed with cancer each year in the United States. Despite the rarity of childhood cancer, there were approximately 12,400 children and adolescents younger than 20 years of age diagnosed with cancer in 1998 in the United States (8,700 cases among children 0 to 14 years of age and 3,700 cases among 15- to 19-year-olds). [1] The likelihood of a young person reaching adulthood and being diagnosed with cancer during childhood is 1 in 300 for males and 1 in 333 for females. [1] Childhood cancer remains the leading cause of disease-related mortality among children 1 to 14 years of age (Fig. 1-1A), and there are 1,500 to 1,600 cancer-related deaths annually in the United States among children younger than 15 years of age. The relative contribution of cancer to overall mortality for 15- to 19-year-olds is less than that for younger children (Fig. 1-1B), although approximately 700 deaths from cancer occur annually in this age group.

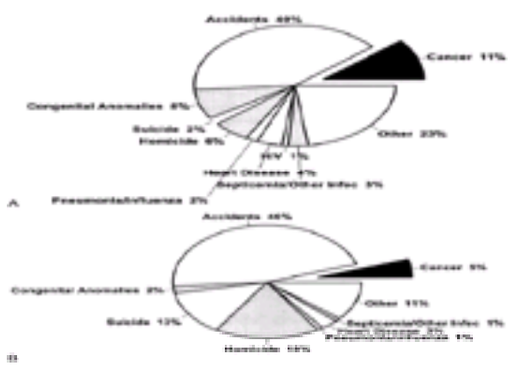


**FIGURE 1-1.** Leading causes of death in children in the United States, 1997. Causes of death among **(A)** children 1 to 14 years and **(B)** adolescents 15 to 19 years of age. (Death data are from the National Center for Health Statistics public-use file.)

The purpose of this chapter is to describe the incidence, survival, and mortality rates for the various types of cancer that arise in children, and to describe how these rates vary by age, race and ethnicity, and gender. Changes in incidence, survival, and mortality over time are also described. The reader is referred to the technical appendix for definitions of the epidemiologic terms used in this chapter. The population-based data for invasive cancer incidence and survival, unless otherwise indicated, are from the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute (NCI). The SEER data for this chapter are based on 24,254 cases of childhood cancer diagnosed among residents of nine SEER areas that represent approximately 10% of the U.S. population. The mortality data cover all cancer deaths among children in the United States, as provided by the National Center for Health Statistics. The classification scheme used in this chapter is the International Classification of Childhood Cancer, which allocates tumors into 12 major diagnostic groups that reflect the most prevalent tumors in the pediatric population. [2]

## OVERALL CANCER FREQUENCY AND INCIDENCE BY TYPE OF CANCER FOR CHILDREN AND ADOLESCENTS

Figure 1-2 compares the distribution by percentages of the cancers that occurred among 0- to 14-year-olds and 15- to 19-year-olds for the years 1990 to 1997, whereas Table 1-1 provides the annual incidence of the major types of cancer in these two age groups. For children 0 to 14 years, acute lymphoblastic leukemia (ALL) was the most common cancer, accounting for 23.5% of all cancer diagnoses. Acute myeloid leukemia (AML) was the next most common type of leukemia in this age group, occurring at a rate one-fifth that for ALL. Central nervous system (CNS) tumors accounted for 22.1% of cancer diagnoses, and together with ALL and AML made up one-half of cancer diagnoses among children younger than 15 years of age. The most common non-CNS solid tumor in the 0- to 14-year age group was neuroblastoma (7.9%), followed by Wilms' tumor (6.0%) and non-Hodgkin's lymphoma (NHL) (5.7%). Other diagnoses that individually represented 2% to 4% of cancer diagnoses in this age group included Hodgkin's disease, rhabdomyosarcoma, non-rhabdomyosarcoma soft tissue sarcomas, germ cell tumors, retinoblastoma, and osteosarcoma.

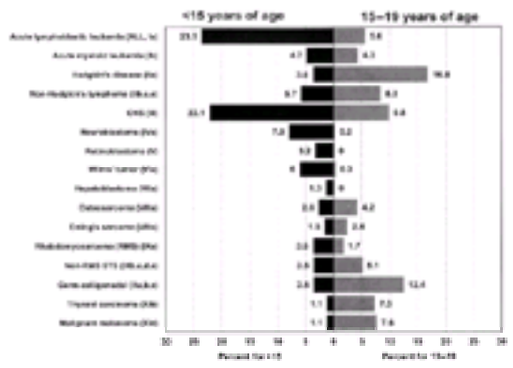


**FIGURE 1-2.** Distribution of specific cancer diagnoses for children (0 to 14 years) and adolescents (15 to 19 years), 1990 to 1997. Percent distribution by International Classification of Childhood Cancer diagnostic groups and subgroups for <15 years and 15 to 19 years of age (all races and both sexes). CNS, central nervous system; RMS, rhabdomyosarcoma; STS, soft tissue sarcoma. (Incidence data are from the Surveillance, Epidemiology, and End Results program, National Cancer Institute.)

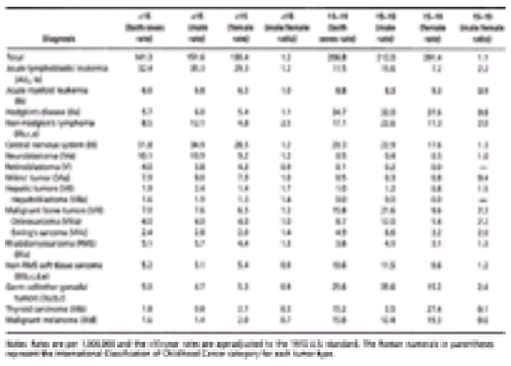

**TABLE 1-1. INCIDENCE OF DIFFERENT CANCERS BY GENDER FOR THE 0- TO 14-YEAR-OLD AND 15- TO 19-YEAR-OLD POPULATIONS (1990–1997)**

A different distribution of cancer diagnoses was observed for 15- to 19-year olds ( Fig. 1-2). Hodgkin's disease (16.8%) and germ cell tumors (12.4%) were the most frequently diagnosed cancers among 15- to 19-year-olds. The percentages of cases represented by NHL (8.3%), melanoma (7.6%), thyroid cancer (7.3%), non-rhabdomyosarcoma soft tissue sarcoma (5.1%), osteosarcoma (4.2%), and Ewing's sarcoma (2.4%) were also higher for 15- to 19-year-olds compared to 0- to 14-year-olds. Although CNS tumors were the third most common tumor type, representing 9.8% of diagnoses (Fig. 1-2), their incidence was lower for 15- to 19-year-olds compared to 0- to 14-year-olds (Table 1-1). ALL accounted for a much lower proportion of cases among 15- to 19-year-olds (5.6%) compared to children 0 to 14 years, and occurred only slightly more frequently than AML (4.3% of cases) in this age group. The percentages for rhabdomyosarcoma and non-rhabdomyosarcoma soft tissue sarcoma were nearly equal for 0- to 14-year-olds, but the percentage for non-rhabdomyosarcoma soft tissue sarcoma was three times higher than that for rhabdomyosarcoma for 15- to 19-year-olds ( Fig. 1-2). Some cancers of young children (e.g., neuroblastoma, retinoblastoma, hepatoblastoma, and Wilms' tumor) occurred at very low rates among 15- to 19-year-olds ( Table 1-1).

### Variation in Childhood Cancer Incidence by Gender

Table 1-1 shows the incidence of cancer by gender for the overall childhood and adolescent population. For both 0- to 14-year-olds and 15- to 19-year-olds, a male predominance was most apparent for NHL, with males having incidence rates more than twice those of females. For children 0 to 14 years of age, other cancer diagnoses that showed a 1.2-fold or higher male predominance were ALL, CNS tumors, neuroblastoma, hepatoblastoma, Ewing's sarcoma, and rhabdomyosarcoma. For 15- to 19-year-olds, the patterns of incidence by gender were generally similar to those observed in younger children, but with the following exceptions:

1. Hodgkin's disease among younger children occurred at a higher incidence among males, whereas among adolescents Hodgkin's disease occurred at a higher incidence among females.
2. For germ cell tumors, females had higher rates among younger children, and males had higher rates among adolescents.
3. Osteosarcoma, which occurred at similar rates in males and females in the 0- to 14-year-old population, occurred at 2.2-fold higher rates in males among 15- to 19-year-olds. Similarly, the male predominance for Ewing's sarcoma was more pronounced in the 15- to 19-year-old group (2.0-fold higher) than in younger children (1.4-fold higher).
4. Thyroid cancer, which was primarily diagnosed among 15- to 19-year-olds, occurred at nearly eight-fold higher rates in females than in males.

### Variation in Childhood Cancer Incidence by Race and Ethnicity

For many adult cancers, black Americans have higher incidence rates than white Americans. For children 0 to 19 years of age, however, the incidence of cancer among white children was approximately 30% higher than that for black children for the years 1990 to 1997 ( Table 1-2; Fig. 1-3). The largest difference in absolute incidence between white children and black children was for ALL (27.8 versus 15.2 per million). This difference was primarily due to the approximately 2.4-fold higher incidence rate for ALL among 0- to 4-year-old white children compared to 0- to 4-year-old black children. [3] The higher rates for leukemia were limited to ALL, as white children and black children had identical rates for AML ( Table 1-2). The incidence of Ewing's sarcoma in white children was nine times higher than that for black children. For melanoma, white children had incidence rates more than 30 times higher than black children ( Table 1-2). White males had much higher rates for testicular germ cell tumors than black males (9.1 versus 1.2 per million), although black females had similar rates of ovarian germ cell tumors as white females (5.6 versus 4.5 per million). [4]

| Cancer type | White | Black | Ratio |
|---|---|---|---|
| Total | 155.9 | 120.8 | 1.3 |
| Acute lymphoblastic leukemia (ALL, Ia) | 27.8 | 15.2 | 1.8 |
| Acute myeloid leukemia (Ib) | 6.1 | 6.1 | 1.0 |
| Hodgkin's disease (IIa) | 15.5 | 9.7 | 1.6 |
| Non-Hodgkin's lymphoma (IIb,c,e) | 10.5 | 7.3 | 1.5 |
| CNS (III) | 27.6 | 22.7 | 1.2 |
| Neuroblastoma (IVa) | 8.0 | 6.7 | 1.2 |
| Retinoblastoma (V) | 2.7 | 3.1 | 0.9 |
| Wilms' tumor (VIa) | 6.2 | 7.1 | 0.9 |
| Hepatoblastoma (VIb) | 1.0 | 0.9 | 1.2 |
| Osteosarcoma (VIIa) | 4.4 | 5.6 | 0.8 |
| Ewing's sarcoma (VIIc) | 3.6 | 0.4 | 9.0 |
| Rhabdomyosarcoma (IXa) | 4.5 | 5.0 | 0.9 |
| Non-rhabdomyosarcoma STS (IXb,c,d,e) | 6.1 | 7.6 | 0.8 |
| Germ cell (Xa,b,c) | 9.3 | 6.1 | 1.5 |
| Thyroid carcinoma (XIIa) | 5.3 | 2.2 | 2.4 |
| Malignant melanoma (XIII) | 5.3 | 0.2 | 35.2 |

CNS, central nervous system tumors; STS, soft tissue sarcoma.
Note: Rates are per 1,000,000 and are age-adjusted to the 1970 U.S. standard. The Roman numerals in parentheses represent the International Classification of Childhood Cancer category for each tumor type.

**TABLE 1-2. INCIDENCE OF DIFFERENT CANCERS FOR WHITE CHILDREN AND BLACK CHILDREN, 0 TO 19 YEARS OLD (1990–1997)**

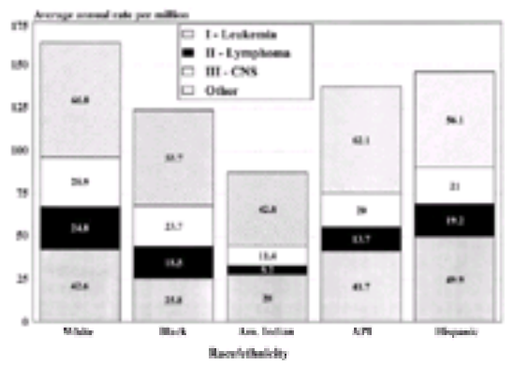


**FIGURE 1-3.** Age-adjusted incidence rates for childhood cancer by race and ethnicity, 1990 to 1997. Data are for International Classification of Childhood Cancer diagnostic groups (age 0 to 19 years and both sexes). Am. Indian, American Indian or Native American; API, Asian/Pacific Islander; CNS, central nervous system; Hispanic, Hispanic of any race and overlaps other categories. (Incidence data are from the Surveillance, Epidemiology, and End Results program, National Cancer Institute, and are adjusted to the 1970 U.S. standard population.)

In contrast to black children, Hispanic children had higher rates of leukemia than white children (49.9 per million versus 42.6 per million) ( Fig. 1-3). However, overall cancer incidence for Hispanic children was lower than that for white children because of lower rates for CNS tumors, lymphomas, and other tumors. The incidence of leukemia was similar for Asian/Pacific Islander children and white children, but Asian/Pacific Islander children had lower rates for CNS tumors and lymphomas.

Overall cancer incidence for American Indian children was much lower than for any other group.

### International Variation in Childhood Cancer Incidence

Figure 1-4 shows the incidence of childhood cancer in selected countries from North America, Europe, Asia, and Australia. [5] Comparisons between countries must be made with caution because of variation in the completeness of cancer registration, as both underdiagnosis and underregistration can lead to inaccurately low estimates of incidence.[5] Given this caveat, cancer incidence was remarkably uniform in many of the countries of Western Europe, North America, Japan, and Australia, with annual incidence for most countries or regions being within 10% to 15% of the incidence observed for white children in the United States. Within these countries, the greatest source of variation was for ALL and for CNS tumors. For ALL, rates for countries shown in Figure 1-4 ranged from 28.4 and 32.8 per million for Japan (Osaka) and the United Kingdom, respectively, to 42.8 and 46.3 per million for Denmark and Costa Rica, respectively. For children living in developing countries, the incidence of childhood ALL appears to be lower than that observed in developed nations. [6,7] The peak in ALL incidence at 2 to 3 years of age observed for children in developed nations (Fig. 1-5B) is absent or greatly diminished for children living in developing nations. [6] For CNS tumors, rates were lower in the United Kingdom and Japan (27 per million for each) and highest in Sweden (41 per million). Differential diagnosis and registration of benign brain tumors and low-grade gliomas may account for a substantial portion of the difference for CNS tumors.

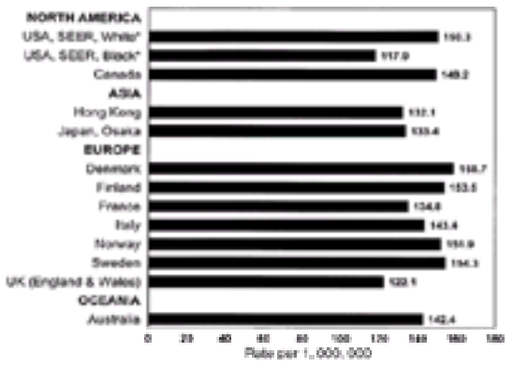


**FIGURE 1-4.** Age-adjusted cancer incidence for children (0 to 14 years). International comparisons. The years included in determining incidence varied for different countries, but generally involved a time period from the early 1980s through the early 1990s. Rates are age-adjusted to the World Standard Population. SEER, Surveillance, Epidemiology, and End Results program. (From Parkin DM, Kramarova E, Draper GJ, et al. International incidence of childhood cancer. Lyon, France: IARC Scientific Publications, 1999.)

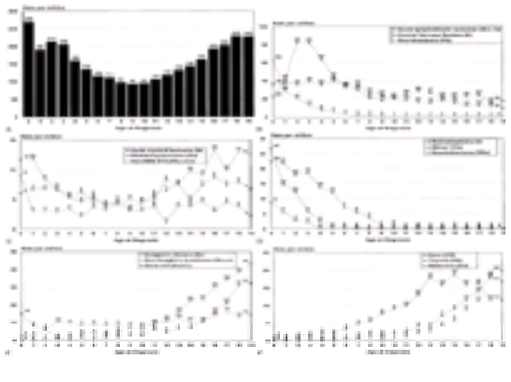

**FIGURE 1-5.** Age-specific incidence rates for childhood cancer by International Classification of Childhood Cancer group (all races and both sexes). Incidence data are from the Surveillance, Epidemiology, and End Results program, National Cancer Institute, and are for the years 1976 to 1984 and 1986 to 1994, unless otherwise indicated. **A:** All cancers (1986 to 1994). **B:** Acute lymphoblastic leukemia (1986 to 1994), brain/central nervous system tumors (1986 to 1994), and neuroblastoma. **C:** Acute myeloid leukemia and non-rhabdomyosarcoma soft tissue sarcomas (non-RMS). **D:** Wilms' tumor, hepatoblastoma, and retinoblastoma. **E:** Hodgkin's disease, non-Hodgkin's lymphoma, and germ cell tumors. **F:** Thyroid carcinoma, melanoma, and bone tumors.

The most dramatic variation in childhood cancer incidence is for those tumors with a clear association to infectious agents. Burkitt's lymphoma, for which one subtype is associated with Epstein-Barr virus infection,[8] represented only a small percentage of cancers diagnosed in most countries, but accounted for more than one-third of childhood cancers registered in some regions of Africa.[5] Similarly, Kaposi's sarcoma was extremely rare in children in most countries where human immunodeficiency virus infection in children is rare,[9] but in some countries of central Africa where human immunodeficiency virus infection is endemic, Kaposi's sarcoma accounted for as much as 15% to 35% of reported cancer cases.[10] Other cancers associated with infectious agents [e.g., hepatocellular carcinoma (hepatitis B virus) and cervical cancer (human papillomavirus)] predominantly manifest in adults, and rates of these infection-related cancers are generally low in children. However, universal hepatitis B vaccination programs reduced the incidence of hepatocellular carcinoma in older children living in a region in which hepatitis B was hyperendemic. [11]

Adrenocortical carcinoma is a rare childhood cancer with an annual incidence of 0.2 per million for U.S. children. [5] In southern Brazil, the annual incidence of adrenocortical carcinoma is approximately 10-fold higher, with rates of 2.8 per million reported by the Goiania Cancer Registry. A very high percentage of children with adrenocortical carcinoma in Brazil have p53 germline mutations, [12] illustrating that differences in childhood cancer incidence between populations may be caused by differences in the concentration of genetically susceptible persons. [13]

### Incidence by Single Year of Age

Childhood cancer incidence is greatest in the first year of life, with a second peak at 2 to 3 years of age, followed by declining rates until age 9 and then steadily increasing rates through adolescence (Fig. 1-5A). Each cancer type has its own distinctive age-distribution pattern.

- ALL incidence peaks at 2 to 3 years of age (Fig. 1-5B).
- In contrast, AML incidence is highest in the first 2 years of life (Fig. 1-5C), with a trough in incidence between age 5 to 9 years, followed by higher rates in adolescence.
- One set of cancers occurs primarily in children younger than 5 years of age. This group includes neuroblastoma, retinoblastoma, Wilms' tumor, and hepatoblastoma (Fig. 1-5B and Fig. 1-5D).
- The incidence of rhabdomyosarcoma is somewhat higher among young children than other age groups (Fig. 1-5C).
- The non-rhabdomyosarcoma soft tissue sarcomas show a peak in incidence in the first year of life (due in large measure to infantile fibrosarcoma), [14] followed by much lower rates until approximately 8 years of age, when rates begin to increase and surpass those for rhabdomyosarcoma (Fig. 1-5C).
- The germ cell tumors also show two peaks in incidence, with the first occurring in infancy and with the second occurring in late adolescence (Fig. 1-5E). The types of germ cell tumors that arise in young children are now known to be biologically distinctive from those that develop in older adolescents. [15]
- A third group of tumors is uncommon among young children and has highest incidence in the 15- to 19-year-age group. This group includes Hodgkin's disease (Fig. 1-5E) and the bone tumors, thyroid cancer, and melanoma (Fig. 1-5F).

### Time Trends for Childhood Cancer Incidence

The incidence of cancer for children 0 to 14 years of age increased by 11.5% between 1975 to 1979 and 1995 to 1997 (125.7 to 140.2 per million) (Table 1-3). Assuming a constant rate of increase in incidence, the estimated annual percentage change (EAPC) in cancer incidence for 1975 to 1997 was 0.7%, but there was

substantial year-to-year variation in rates ( Fig. 1-6A). For example, the incidence varied from 132 per million in 1988 to 148 per million a year later and back down to 134 per million in 1992. The increase in incidence for children 0 to 14 years of age between 1975 and 1997 appears to have primarily occurred before 1985, with incidence for 1995 to 1997 being essentially the same as that for 1985 to 1989, and with the EAPC for 1985 to 1997 being 0.0%.

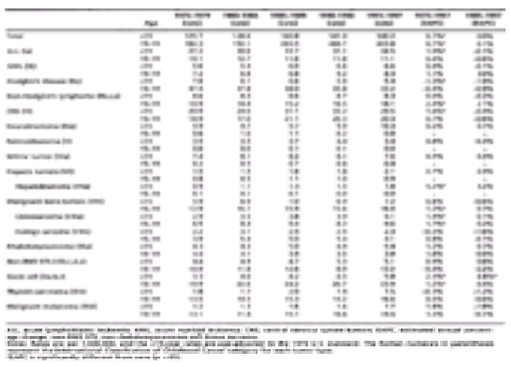

**TABLE 1-3. INCIDENCE OF CHILDHOOD AND ADOLESCENT CANCERS BY 5-YEAR TIME PERIODS AND TRENDS IN INCIDENCE (1975–1997 AND 1985–1997)**

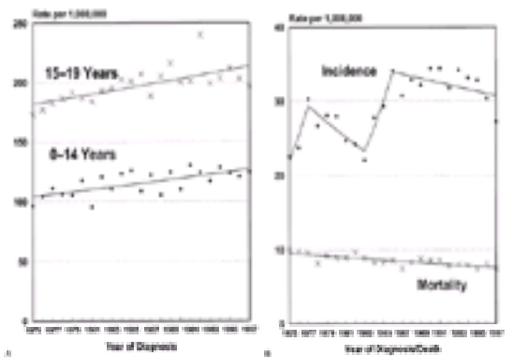


**FIGURE 1-6.** Trends in childhood cancer incidence over time. **A:** Surveillance, Epidemiology, and End Results (SEER) program incidence for all childhood cancers, age 0 to 14 years and age 15 to 19 years (all races and both sexes) for 1975 to 1997. **B:** SEER incidence and U.S. mortality for brain and central nervous system tumors, age 0 to 14 years (both sexes and all races), 1975 to 1997. Rates are age-adjusted to the 1970 U.S. standard million population. Regression lines are calculated using the Joinpoint Regression Program. (Incidence data are from the SEER program, National Cancer Institute. Mortality data are from National Center for Health Statistics.)

The increase in cancer incidence from 1975 to 1979 and 1995 to 1997 for children 0 to 14 years of age resulted primarily from increases in the incidence of CNS tumors and from increases in the incidence of ALL. The increase in childhood CNS tumor incidence did not occur at a steady rate, but instead abrupt increases in incidence occurred in the late 1970s and the mid-1980s, followed by stable or declining rates ( Fig. 1-6B). The increases occurred during periods in which advances in diagnostic imaging for CNS tumors were becoming widely disseminated (computed tomographic imaging in the mid- to late 1970s and magnetic resonance imaging in the mid-1980s).[16,17] The incidence for childhood CNS tumors has not increased since the widespread availability of magnetic resonance imaging in the mid-1980s (EAPC = –0.4% for 1985 to 1997).[18] The periods of abrupt changes in incidence were not associated with changes in CNS tumor mortality rates, which slowly decreased throughout the entire period from 1975 to 1997 ( Fig. 1-6B). The timing of the increases in childhood CNS tumor incidence and the lack of corresponding increases in mortality rate suggest that improvements in diagnosis or reporting, or both, of childhood CNS tumors may account for much of the increase.

Childhood ALL incidence increased from 27.2 per million in 1975 to 1979 to 34.5 per million in 1995 to 1997, but most of the increase occurred between 1975 and 1984. From 1985 to 1997, incidence for ALL was essentially stable (EAPC = –0.1%), although there were unexplained wide variations in year-to-year incidence, with a low of 29 per million in 1994 and a high of 38 per million in 1989. For most of the remaining cancers, incidence remained relatively constant from 1975 to 1997 for children younger than 15 years ( Table 1-3), with only the expected year-to-year variation related to the relatively small numbers of cases of these tumor types diagnosed annually. Statistically significant increases in incidence were observed for hepatoblastoma, osteosarcoma, and germ cell tumors, whereas a significant decrease in incidence was observed for Hodgkin's disease.

For the 15- to 19-year-old population, the annual incidence of cancer increased from 182.3 per million for 1975 to 1979 to 203.8 per million for 1995 to 1997. This represented a significant increase in incidence for the period 1975 to 1997 (EAPC = 0.7%), although there was substantial year-to-year variation in rates ( Fig. 1-6A). Incidence for 1995 to 1997 was essentially identical to that for 1985 to 1989, and the EAPC for 1985 to 1997 was 0.1%. NHL was the largest contributor to the increase in incidence for 15- to 19-year-olds, with incidence rising from 10.9 per million in 1975 to 1979 to 18.1 per million in 1995 to 1997 ( Table 1-3). This increase in NHL incidence for 15- to 19-year-olds, which was not observed for 0- to 14-year-olds, is similar to the increase in NHL incidence that occurred during the same period for adults.[19,20] Significant increases in incidence were also observed for osteosarcoma and for germ cell tumors ( Table 1-3).

## SURVIVAL AND MORTALITY RATES FOR CHILDREN WITH CANCER

Survival rates for children 0 to 14 years of age have improved dramatically since the 1960s when the overall 5-year survival rate after a cancer diagnosis was estimated as 28%.[21] Improvements in survival rates have continued into the 1990s in the United States ( Fig. 1-7), with 3-year survival rates exceeding 80% and 5-year survival rates exceeding 75% for children and adolescents diagnosed during this period ( Fig. 1-7).

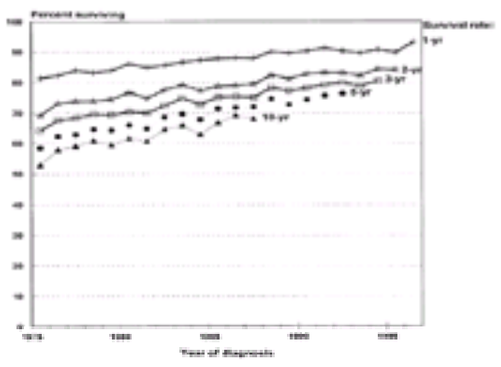

**FIGURE 1-7.** Trends in relative survival rates for all childhood cancers, age 0 to 19 years (all races and both sexes) for Surveillance, Epidemiology and End Results (SEER) program regions (nine areas), 1975 to 1996. (Data are from the SEER program, National Cancer Institute.)

The increase in survival for children younger than 15 years of age was most dramatic for ALL, a virtually incurable disease in the early 1960s and for which 5-year survival rates exceeded 80% in 1989 to 1996 (Fig. 1-8A). Survival rates for childhood NHL increased to nearly 80% in 1989 to 1996, up from 20% to 25% in the early

1960s (Fig. 1-8B), and survival rates for Wilms' tumor increased from 33% to 92% during the same period ( Fig. 1-8B). Five-year survival rates at or above 90% have also been achieved for Hodgkin's disease, retinoblastoma, thyroid cancer, and melanoma ( Fig. 1-9). Five-year survival rates for bone tumors (Fig. 1-8B) and for CNS tumors (Fig. 1-8A) increased to 68% and 65% by 1989 to 1996, respectively, whereas 5-year survival rates for AML remained below 50% ( Fig. 1-8A).

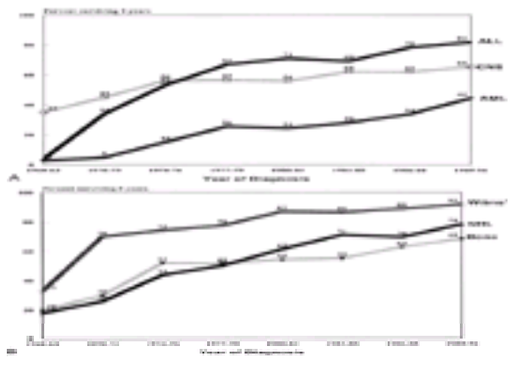

**FIGURE 1-8.** Five-year relative survival rates for specific cancers of children (0 to 14 years) in 1960 to 1996. Data for 1960 to 1963 and for 1970 to 1973 are from the End Results Group at the National Cancer Institute (NCI) and are for white children [21]; 1974 to 1996 data are from the Surveillance, Epidemiology, and End Results (SEER) program regions (nine areas). **A:** ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CNS, central nervous system. **B:** Bone tumors; NHL, non-Hodgkin's lymphoma; and Wilms' tumor.

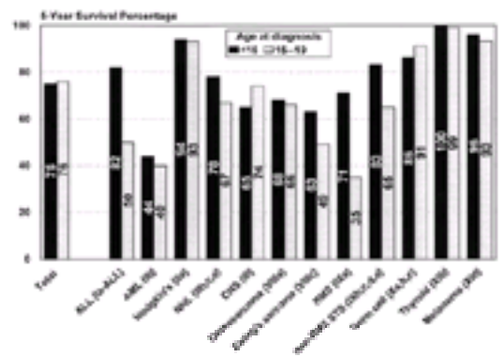


**FIGURE 1-9.** Survival for 0- to 14-year-olds and for 15- to 19-year-olds in Surveillance, Epidemiology, and End Results (SEER) program regions (nine areas), 1989 to 1996. Rates are for all races and both sexes. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CNS, central nervous system; NHL, non-Hodgkin's lymphoma; non-RMS, non-rhabdomyosarcoma; RMS, rhabdomyosarcoma. (Data are from the SEER program, National Cancer Institute.)

Survival rates for 15- to 19-year-olds were similar to those for younger children for most cancer types, including brain tumors, NHL, osteosarcoma, Hodgkin's disease, Ewing's sarcoma, AML, and germ cell tumors (Fig. 1-9). Survival rates for 15- to 19-year-olds with ALL were lower than those for younger children, which could be due in part to a higher proportion of cases with unfavorable biology among 15- to 19-year-olds. A similar explanation may explain the lower survival rates for 15- to 19-year-olds with rhabdomyosarcoma and non-rhabdomyosarcoma soft tissue sarcoma, and the higher survival rates for 15- to 19-year-olds with CNS tumors. Survival rates above 90% were observed for four of the most common cancers among 15- to 19-year-olds: Hodgkin's disease, germ cell tumors, thyroid cancer, and melanoma.

Cancer mortality rates have decreased for children since the 1950s as a result of improvements in treatment and the resulting increased survival rates. In the 1950s, childhood cancer mortality rates were stable at approximately 80 per million ( Fig. 1-10A). The cancer mortality rate for 0- to 19-year-olds began declining in the 1960s and by the late 1990s had decreased to less than 30 per million. Declines in mortality for the leukemias began in the early 1960s, with rates decreasing from 30 to 35 per million to less than 10 per million by the late 1990s ( Fig. 1-10A). For NHL, declining mortality began in the late 1960s, with rates decreasing from 6 to 7 per million to less than 2 per million by the 1990s (Fig. 1-10B). Mortality from kidney tumors (primarily Wilms' tumor) decreased by 80% over a similar time period from approximately 4 per million to less than 1 per million. Mortality rates also declined for Hodgkin's disease (not shown), with rates decreasing from approximately 3 per million in the 1950s and early 1960s to approximately 0.4 per million in the mid-1990s. The brain cancer mortality rate was approximately 10 per million in 1970 and had decreased to approximately 7 per million by 1997 (Fig. 1-10B).

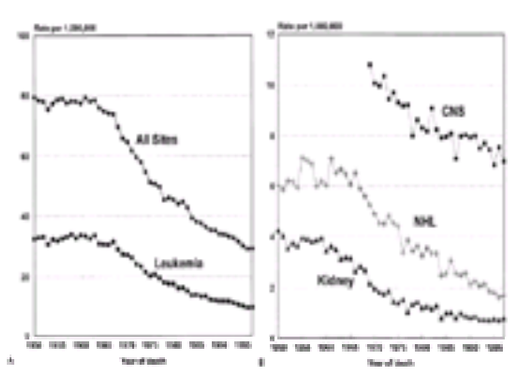


**FIGURE 1-10.** Mortality rate for children and adolescents 0 to 19 years in the United States, 1950 to 1997. **A:** Mortality rates for all cancers and for leukemia. **B:** Mortality rates for non-Hodgkin's lymphoma (NHL), brain/other nervous system (ONS) tumors, and Wilms' tumor. Death data are from the National Center for Health Statistics public-use file. Death rates for brain/ONS tumors are restricted to the years 1969 to 1997 because the coding of brain/ONS tumors in the International Classification of Diseases (ICD), Seventh Revision is not compatible with ICD-8 and -9 (used from 1969 forward). CNS, central nervous system.

Figure 1-11 shows the distribution of causes of cancer death for 0- to 19-year-olds in 1997. Approximately one-third of cancer-related deaths were caused by leukemias, with ALL accounting for an estimated 50% to 60% of deaths, AML for 30% to 40% of deaths, and CML for approximately 5% of deaths. CNS tumors were the second leading cause of cancer mortality among children and adolescents, accounting for 24% of cancer-related deaths. The other primary causes of cancer-related mortality were neuroblastoma (classified under endocrine tumors), bone tumors, soft tissue sarcomas, and NHL.

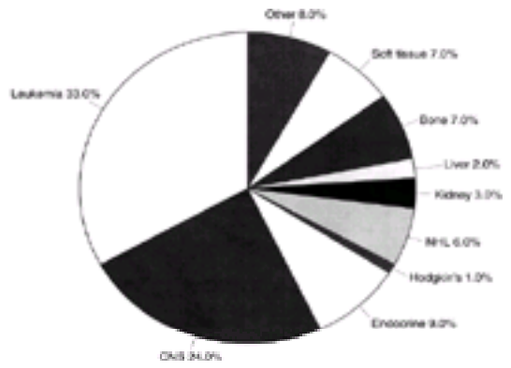


**FIGURE 1-11.** Percent distribution by cause of cancer death in children and adolescents 0 to 19 years. Death data are from the National Center for Health Statistics public-use file. The endocrine category primarily represents neuroblastoma. CNS, central nervous system; NHL, non-Hodgkin's lymphoma.

## SUMMARY

The cancers of children represent a diverse group of diagnoses that have distinctive age-incidence patterns. Even within a single diagnosis, the biologic characteristics of tumor cells may vary between younger and older children. Interpreting changes over time in incidence, survival, and mortality rates for childhood cancers requires consideration of the distinctive age-incidence profiles of the cancers that arise in children.

The incidence of cancer among children 0 to 14 years and among 15- to 19-year-olds increased on average by less than 1% per year between 1975 and 1997. Since the mid-1980s, overall cancer incidence has been essentially stable for both age groups. The increase in incidence for 0- to 14-year-olds was primarily due to increases in incidence for CNS tumors and leukemias (primarily ALL), with increases also observed for osteosarcoma, germ cell tumors, and hepatoblastoma. For 15- to 19-year-olds, increasing incidence was observed for NHL, osteosarcoma, and germ cell tumors. Rates for Hodgkin's disease decreased in both age groups.

Childhood cancer mortality rates decreased dramatically between 1960 and 1997, with decreases in mortality observed for all cancer types. These decreases are a tribute to the clinical investigators who for decades have diligently collaborated to conduct clinical trials that have identified improved treatments for children with cancer. In spite of these remarkable advances, for approximately 25% of children and adolescents diagnosed with cancer, current treatments are not sufficiently effective to allow cure, and more than 2,000 children and adolescents still die of cancer each year in the United States. Identifying curative treatments for these children will require deeper understandings of the mechanisms responsible for survival and growth of specific pediatric cancers and will require new treatment approaches based on these understandings. Given the relative rarity of specific childhood cancers, continued national and international cooperation will be essential for identifying superior treatments that build on an ever-increasing understanding of the biology of childhood cancers.

## TECHNICAL APPENDIX

**Age-adjusted rate** An age-adjusted rate is a weighted average of the age-specific cancer incidence (or mortality) rates, where the weights are the proportions of persons in the corresponding age groups of a standard population. The potential confounding effect of age is reduced when comparing age-adjusted rates computed using the same standard population. Because rates of childhood cancer vary widely by 5-year age group, age-adjustment was used for any age group representing more than one 5-year grouping. Age-adjustment was performed by 5-year age group and weighted by the 1970 U.S. standard million population except for the comparison of international rates, which was age-adjusted to the world standard.

**Age-specific rates** Age-specific rates are usually presented as a rate per million for childhood cancer. The numerator of the rate is the number of cancer cases found in a particular 5-year age group in a defined population divided by the number of individuals in the same 5-year age group in that population. In this publication, there are some rates by single year of age for time periods around the census. Population estimates by single year of age, race, sex, and geographic region are not generally available for intercensal years.

**Estimated annual percentage change (EAPC)** The EAPC was calculated by fitting a regression line to the natural logarithm of the rates (r) using calendar year as a regressor variable (i.e., $y = mx + b$ where $y = \text{Ln } r$ and $x$ = calendar year). The $EAPC = 100*(e^{m} - 1)$. Testing the hypothesis that the EAPC is equal to zero is equivalent to testing the hypothesis that the slope of the line in the above equation is equal to zero. The latter hypothesis is tested using the t distribution of $m/SE_m$ with the number of degrees of freedom equal to the number of calendar years minus two. The standard error of m (i.e., $SE_m$) is obtained from the fit of the regression.[22] This calculation assumes that the rates increased/decreased at a constant rate over the entire calendar year interval. The validity of this assumption has not been assessed. In those few instances where at least one of the rates was equal to zero, the linear regression was not calculated.

**Follow-up** SEER areas attempt to follow-up all cases until death. Although the overall proportion of cancer patients of all ages who are lost to follow-up is only approximately 5%, for pediatric cases (age 0 to 19) it is much larger—approximately 14%. Because survival rates are relatively high, follow-up can be difficult, especially as the child gets older. When children leave their parents' home, they change addresses and, especially for females, they may change last names.

**ICCC classification** At the time the World Health Organization's International Agency for Research on Cancer published their first monograph on Childhood Cancer in 1988, Dr. R. Marsden published an annex giving a classification scheme for childhood cancer that consisted of 12 groups based chiefly on histologic type. [23] The classification by Marsden has been modified and is now called the *International Classification of Childhood Cancers*.[2]

**Incidence rate** The cancer incidence rate is the number of new cancers of a specific site/type occurring in a specified population during a year, expressed as the number of cancers per 1 million people. It should be noted that the numerator of the rate can include multiple primary cancers occurring in one individual. This rate can be computed for each type of cancer as well as for all cancers combined. Except for 5-year age-specific rates, all incidence rates are age-adjusted to the 1970 U.S. standard million population. Rates are for invasive cancer only, unless otherwise specified.

**Mortality data** The mortality data are from public use files provided by the National Center for Health Statistics and cover all deaths in the United States. All mortality rates were based on the underlying cause of death. The rates presented for 1975 to 1978 were coded to the *International Classification of Diseases (ICD), Eighth Revision* and for 1979 to 1995 to the *ICD-9*.[24] Certain groups can be identified as specific entities on death certificates: leukemias, bone cancers, brain and other CNS tumors, Hodgkin's disease, and NHL. However, mortality of all specific groups of the ICCC pediatric classification are not available from U.S. mortality files due to the fact that the codes used for coding death certificates do not include such morphologic types as neuroblastoma, retinoblastoma, germ cell tumors, and Wilms' tumor. For neuroblastoma, to make the data comparable over time, deaths coded to sympathetic nervous system in the *ICD-8* were combined with adrenal in the *ICD-9* and are included in the endocrine tumor category.

**Mortality rate** The cancer mortality rate is the number of deaths with cancer given as the underlying cause of death occurring in a specified population during a year, expressed as the number of deaths due to cancer per 1 million people. This rate can be computed for each type of cancer as well as for all cancers combined. Except for age-specific rates, all mortality rates are age-adjusted to the 1970 U.S. standard million population.

**Population data** Population estimates are obtained each year from the U.S. Bureau of the Census (BOC) at the county level by 5-year age group (0 to 4, 5 to 9, etc., to 85 and older), sex, and race (including white and black). SEER makes county estimates for each state available on the SEER Home Page (http://www.seer.cancer.gov/) for race (whites, blacks, non-white), 5-year age group, sex, and year of diagnosis (each year from 1973 forward). Additional estimates can be obtained from the U.S. Census Bureau Home Page. BOC population estimates for Hawaii were altered according to independent estimates developed from sample survey data collected by the Health Surveillance Program (HSP) of the Hawaii Department of Health. For Hawaii, the all races and black populations are the same as those sent by the BOC. Proportions of the population by different racial groups from the HSP were used to generate estimates for whites, and so forth. Because the HSP survey was for all of Hawaii and not by county, population estimates were not broken down by county. The white population estimates for Hawaii provided by the BOC are generally larger than those generated by the HSP. Because whites in Hawaii account for less than 2% of the total white population represented by the SEER reporting areas, white incidence rates for the entire SEER program are not noticeably affected. Procedures for calculating rates by race for

Hawaii are currently under review.

**Primary site/histology coding** Originally, data for site and histologic type were coded by the *ICD for Oncology* (*ICD-O*), but in 1990, *ICD-O* was revised and republished as the *ICD-O, Second Edition* (*ICD-O-2*).[25] SEER areas began using *ICD-O-2* for cases diagnosed in 1992 and machines converted all previous data to *ICD-O-2*. Most data for NHL can be classified by the Working Formulation based on a conversion from *ICD-O-2*.

**Relative survival rate** The relative survival rate is calculated using a procedure described by Ederer, Axtell, and Cutler whereby the observed survival rate is adjusted for expected mortality.[26] The relative survival rate represents the likelihood that a patient will not die from causes associated specifically with their cancer at some specified time after diagnosis. It is always larger than the observed survival rate for the same group of patients.

**SEER program** This program started in 1973, as an outgrowth of the NCI's Third National Cancer Survey and the End Results program. NCI contracts out with various medically oriented non-profit organizations, local city or state health departments, or universities for collection of these data. Contracts for collecting this data are with the entire states of Connecticut, Iowa, New Mexico, Utah, and Hawaii and with the metropolitan areas of Los Angeles, California; Detroit, Michigan; San Francisco-Oakland and San Jose-Monterey, California; Seattle-Puget Sound, Washington; and Atlanta, Georgia. These organizations collect data on all cancers except basal and squamous cell skin cancers and *in situ* of the cervix uteri. Although data are collected on all people having cancer, the material for this chapter used children from birth through age 19 years. To calculate long-term trends, only nine SEER areas (which together represent approximately 10% of the U.S. population) were used for this chapter: San Francisco-Oakland, California; Seattle-Puget Sound, Washington; Atlanta, Georgia; Detroit, Michigan; Hawaii; Connecticut; Utah; Iowa; and New Mexico. Only residents of the areas designated are included so that the base populations can be properly determined.

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2

# EPIDEMIOLOGIC RESEARCH METHODS AND CHILDHOOD CANCER

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MELISSA L. BONDY



## INTRODUCTION

This chapter provides an overview of epidemiologic methods, including study designs, potential biases, and statistical measures of effect, with examples from the literature to illustrate the concepts. Information in this chapter should help clinicians better understand the approaches used in epidemiologic research on the causes and consequences of childhood cancer, and to interpret and communicate research findings to their patients.

## CENTRAL CONCEPTS OF EPIDEMIOLOGY

Epidemiology, a scientific methodology for conducting health-related research, can be defined as the comparative study of the distribution and determinants of disease and other health-related conditions within defined human populations. Identifying, describing, and interpreting patterns of cancer occurrence (distribution), and studying factors that may cause or contribute to the occurrence, prevention, control, and outcome of cancer (determinants), encompass these two activities. [1,2] Historically, epidemiology strove to identify and control sources of infectious diseases and outbreaks, but the focus in modern times, especially in industrialized countries, now includes chronic diseases such as cancer. Epidemiologic studies in the 1950s on smoking and lung cancer were instrumental in developing the study designs and statistical methodologies used today in childhood cancer research.

Epidemiology incorporates aspects of research from biologic, clinical, social, and statistical sciences. Two central concepts of epidemiology are

1. *Disease is not randomly distributed.* Measurable factors influence the patterns and causes of disease within a defined population.
2. *Disease causation is multifactorial.* Few individual agents are necessary or sufficient to cause disease. Disease results from a multitude of endogenous and exogenous factors. Identifying and measuring the relative contribution and interaction of these factors is the principal role of analytic epidemiology.

## SURVEILLANCE AND DESCRIPTIVE STUDIES

Public health surveillance involves the systematic collection, analysis, and interpretation of outcome-specific health data, and timely dissemination to prevent and control disease or injury. Surveillance systems are thus essential to plan, implement, and evaluate public health practice. [3] Surveillance systems provide data on disease incidence and mortality on a population basis for policy makers and researchers. In the United States, an exceptionally high quality cancer surveillance system, begun in 1973, is funded and coordinated by the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program through contract with five state and six large metropolitan cancer registries ( http://www-seer.ims.nci.nih.gov/). The SEER registry collects cancer incidence information on approximately 14% of the U.S. population and currently includes more than 30,000 cases of malignant neoplasms among children age 19 years and younger. These data serve as the primary source of our understanding of the distribution of and trends in childhood cancer in the United States. The impact of SEER data can be seen in the national debate that followed a report of upward trends in childhood cancer incidence rates, including an average increase for brain cancer of approximately 2% per year.[4] Many papers have since been published to discuss whether changing environmental exposures or changes in diagnostic practices and morphologic coding occasioned the increase. Although some disagree, evidence supports the growing consensus that diagnostic technological advances and revisions in histopathologic categorization of brain tumors explain the higher rates. [5]

Because SEER ascertains and describes virtually every new case of cancer in its reporting area, the population-based nature of the data offers information on the entire disease spectrum, and is thus more representative than individual hospital data, which is limited by referral patterns, specialized patient populations, or small case sizes. For epidemiologists, an additional benefit of population-based registries is that, unlike clinical data, researchers can clearly delineate the populations that gave rise to the cases and use them for calculating reliable incidence rates.

SEER information, too, enables descriptive evaluation, otherwise unachievable, of rare childhood malignancies and of cancer patterns in demographic subgroups. Descriptive analyses from cross-sectional (prevalence) or ecologic (correlational) studies allow investigators to develop hypotheses on the patterns and causes of cancer, permitting assessment by analytic approaches.[1,2] Importantly, the individual cancer registries may, under carefully controlled conditions, allow researchers to contact persons in the database to invite them to participate in analytic studies of cancer etiology. The rarity of any specific type of childhood cancer, however, makes it very difficult to recruit enough cases for statistically meaningful studies, even with statewide population-based registries. This problem of conducting good epidemiologic research on rare events has prompted the newly merged Children's Oncology Group to discuss with the National Cancer Institute the development of a nationwide, population-based childhood cancer registry. Such a national registry would even more effectively arm research efforts into the causes and consequences of childhood cancer.

## ANALYTIC STUDY DESIGNS

Some epidemiologic studies, such as randomized intervention trials and randomized controlled clinical trials, follow the principles of scientific experimentation in which a treatment or intervention of interest and the control condition are randomly assigned. [6] The childhood cancer clinical trials compare one treatment regimen to another, such as the recent study of intensive chemotherapy with or without autologous bone marrow transplantation for high-risk neuroblastoma. This national study from the Children's Cancer Group showed a survival benefit from adjuvant 13-*cis*-retinoic acid among patients without disease progression in both primary treatment arms.[7] Despite some beliefs to the contrary, well-designed and well-conducted non-experimental (observational) studies also can provide accurate estimates of treatment effects.[6,8,9]

Non-experimental analytic studies assess the causal influence of potential risk factors unable to be evaluated experimentally because the experiment would be unethical or impractical. An obviously unethical experiment would, for example, randomize pregnant mothers to ingesting different kinds and amounts of organophosphate pesticides to measure subsequent incidence rates of non-Hodgkin's lymphoma in their offspring. It would be impractical, even if ethical, to randomly allocate newly pregnant mothers to receive high daily doses of vitamins C and E to weigh their efficacy in preventing childhood brain cancer. To provide an accurate and reliable conclusion, the trial would require thousands, if not hundreds of thousands, of preconceptual mothers and their children to be followed for many years. Thus, epidemiologists must use several non-experimental, or observational, study designs to identify causal risk factors and quantify the contribution the risk factors

have on disease incidence on populations with “naturally” occurring exposures varied enough to be useful in comparisons. An example is an international childhood brain cancer study that found evidence to suggest a protective effect of vitamin supplementation during pregnancy. [10] Cohort studies and case-control studies are two analytic observational approaches commonly used by epidemiologists.

### Cohort Studies

Cohort studies evaluate subjects initially free of a specific disease of interest and whose exposure status can be classified. Subjects are followed for a defined time period to ascertain endpoints, such as new events in or death from disease. The disease rate in the exposed group is then compared statistically to the rate in the unexposed group. A prospective cohort study resembles a clinical trial, but subjects are not randomly allocated to an exposure arm. Rather, as mentioned previously, exposure (or lack of exposure) occurs “naturally” and the investigator uses variations in natural exposure levels to evaluate differences in the risk of subsequent disease occurrence during some follow-up period.

Cohort studies permit efficient study of relatively common diseases with a reasonably short latency period from exposure to disease onset. Cohort studies are usually impractical for rare diseases, such as childhood cancer, as statistically meaningful results could be achieved only by assembling and following for a very long time a huge number of at-risk subjects. One notable exception, however, was a cohort of 15,895 Japanese children who were in Hiroshima or Nagasaki at the time of the atomic bombing during World War II, were younger than age 10 years during the bombings, and who survived to at least October 1, 1950 (survived 5 years or longer). As part of a study on the health effects of the atomic bombing victims using a detailed and complicated exposure reconstruction procedure, [11] each child's radiation dose was estimated. With follow-up to 1985, children with a dose of greater than 1 Gy had a cumulative cancer death rate of approximately 26 per 1,000, compared with 6.5 per 1,000 among those with a dose of 0.1 Gy or less.[12] The ratio of these rates, 4.0, is a type of relative risk (described below) and a measure of how strong is the association between ionizing radiation exposure and death from cancer. The study, that is, found a four-fold higher cumulative cancer death rate for those children exposed to higher compared with lower levels of ionizing radiation.

Cohort studies can involve active follow-up of subjects in real time (prospective) like clinical trials, or can be retrospective. Retrospective cohort studies use historical records to identify the study population and to reconstruct their exposure and subsequent disease experience. An example was an evaluation comparing three large birth cohorts to determine if contamination of the Salk poliovirus vaccine with simian virus 40 (SV-40) resulted in an excess of cancer incidence among those exposed. One birth cohort was (inadvertently) exposed to the contaminated vaccine during infancy (born 1956 to 1962), one was exposed later in childhood (born 1947 to 1952), and one was unexposed to SV-40 (born 1964 to 1969). Using cancer registries and mortality records, age-specific cancer incidence rates were calculated for each study group. No meaningful differences in cancer rates overall, or for any specific type of malignancy, were found among the three cohorts. [13]

The current Childhood Cancer Survivors Study includes both retrospective and prospective components. This cohort study identified and recruited more than 14,000 childhood cancer survivors (or their parents for those deceased) from a consortium of 25 medical centers. Eligible subjects survived at least 5 years after diagnosis between 1970 and 1986. To evaluate medical late effects and psychosocial outcomes as a function of treatment, researchers are assembling information from treatment records, telephone interviews, follow-up questionnaires, and buccal cells (for DNA analysis). This study addresses the important question of the long-term consequences of childhood cancer and its treatment among survivors. [14]

### Case-Control Studies

For rare occurrences, such as childhood cancer, case-control studies provide a strategy more efficient than cohort studies to evaluate potential causal associations. A childhood cancer case-control study identifies and recruits children (or their parents) who are diagnosed within a defined population and time period. A similar group of children without the disease, but from the same defined population (in time, location, and eligibility criteria) that gave rise to the cases, are recruited to serve as controls. The investigator, as completely and accurately as possible, uses self-report, health records, and biologic specimens to reconstruct the cases' pre-diagnosis exposure experience. Similarly, a “reference” date substituting for a diagnosis date is assigned to each control child, whose exposure experience before that date is reconstructed. The exposure frequency among the case group is then compared statistically to the exposure frequency among the control group. The resultant statistic, known as an *odds ratio* (OR), is analogous to a relative risk and is a measure of the strength of the association between the exposure and the disease.

Examples include a case-control study in the Seattle area that explored the risk of childhood brain cancer from residential sources of extremely low-frequency electromagnetic fields (EMFs), including nearby power lines, among 120 children diagnosed with brain cancer and 240 control children. [15] The study included personal interviews and mail-in questionnaires from parents, and construction of scaled maps of power distribution systems for coding of EMF exposure. The odds of a case having been exposed to high current power lines was nearly identical to the control children (OR = 0.9) (i.e., the study observed no association between high EMF exposure and childhood brain cancer). Protective associations were found for breast-feeding in two case-control studies of childhood acute leukemia conducted through the Children's Cancer Group. Relying primarily on telephone interviews with mothers for information, investigators compared the breast-feeding frequency of 1,744 children with acute lymphoid leukemia (ALL) to 1,879 control children, and 456 children with acute myeloid leukemia (AML) to 539 control children. Breast-feeding was found associated with a reduced risk for ALL (OR = 0.80) and AML (OR = 0.77) and longer duration of breast-feeding strengthened the apparent protective effect.[16]

### Cluster Investigations

It is not uncommon for clinicians to encounter parental concern about multiple cancer occurrences in their child's community. The implication, of course, is that a shared environmental exposure is responsible for the cluster of cancer cases. Cluster investigations use standard epidemiologic study designs, primarily case-control studies, to ascertain whether an unusual number of cancer cases occurred in a specific area (spatial cluster) or time (temporal cluster) or both (space-time cluster).[17,18] The latter, for instance, would be an excess of childhood leukemia in a neighborhood or school over a specific time period. Public health agencies have the responsibility to investigate cancer clusters and communicate findings to the public. [17] Clinicians are well advised to refer cluster inquiries to local health departments or the Centers for Disease Control and Prevention (http://www.cdc.gov/ or http://www.atsdr.cdc.gov/). Such investigations, however, rarely produce evidence that a true childhood cancer cluster exists. [19]

## MOLECULAR EPIDEMIOLOGY

Classical or traditional epidemiology, as discussed above, permits epidemiologists to evaluate risks and causal roles of environmental factors in cancer. Molecular epidemiology, a hybrid of epidemiology and molecular genetics, enables researchers to assess biologic characteristics that may influence cancer susceptibility. The concept that risk of cancer from a given exposure differs between subgroups of a population is known in the epidemiologic vernacular as *effect modification*; biostatisticians often refer to this heterogeneity of effect as *interaction*. With the advent of polymerase chain reaction and other advanced laboratory methods, epidemiologists can incorporate molecular markers into their studies to identify specific suspect endogenous or exogenous host factors at the biochemical or molecular level.[20] Such studies aim to determine the roles, including interactions, of environmental and genetic factors in the initiation and progression of the carcinogenic process. The approach of incorporating genetic markers in epidemiologic studies of childhood cancer etiology shows promise for reducing cancer risk and providing strategies for prevention. Molecular epidemiology is certainly accompanied by challenges, however, such as ensuring the appropriate interpretation of molecular testing and resolving associated ethical, legal, and social concerns.

From molecular epidemiology has come the identification of biomarkers that may provide information on the extent of exposure to carcinogens. Perera and Weinstein[21] delineated four categories of biomarkers that help predict risk: internal dose, biologically effective dose, response, and susceptibility. Biomarkers represent a valuable research tool for detecting early changes caused by exposures, and they identify individuals with particularly high risk of cancer development. Describing and determining the occurrence of suitably selected biomarkers has led to tremendous progress in research on the mechanisms of cancer initiation and promotion, and has begun to make possible the assessment of cancer risk in healthy individuals. The knowledge that gene defects (gene mutations and changes in their expression) underlie carcinogenesis has resulted in focused efforts to detect such aberrant genes and their associated proteins.

The addition of molecular parameters to population-based studies should help identify genes and pathways involved in cancer development due to environmental exposures and to identify susceptible or resistant subpopulations. In turn, information about molecular mechanisms of carcinogenesis should improve risk assessment. Although studies of childhood cancer are currently limited to only a few candidate genes, the exponential growth of scientific technology and information promises future expansion of knowledge about the identity of potential genes and cancer pathways.

The crux of childhood cancer studies of etiology, in addition to identifying causal factors, is determining the critical period of exposure and disease susceptibility.

Exposures *in utero* and during the early years of life can disproportionately increase risk of cancer later in life. [22,23] Laboratory and epidemiologic evidence suggests that differential exposure response or physiologic immaturity raises the risk for infants and children far above that of adults experiencing the same environmental insults. The underlying mechanisms combine to proportionately increase exposure to toxicants and lessen the ability of the child in early stages of development to detoxify or repair damage. The cancer can be initiated *in utero,* with subsequent genetic mutational events and clonal progression occurring later. Adolescence and young adulthood are also sensitive times because of such proliferative surges as hormone outflow and rapid bone growth.

Current studies of molecular epidemiology are based on an understanding of the complex, multistage process of carcinogenesis and heterogeneous responses to carcinogenic exposures. Quantitative methods to measure human exposures to carcinogens improve continuously and have been successfully applied in a number of epidemiologic studies. Genetic predispositions to cancer, both inherited and acquired, have been, and continue to be, identified. The combined approach of correlating genetic polymorphisms with other cancer risk factors is showing considerable promise. For instance, glutathione S-transferases (GST) enzyme activity is involved in the detoxification of carcinogens such as epoxides and alkylating agents. GST genes are polymorphic, and lack of enzymatic activity potentially increases cancer risk. GST null genotype was hypothesized to increase risk of childhood AML and myelodysplasia (AML/MDS) in a recent case-control study of 292 children with AML/MDS. The frequency of GSTM1 null was significantly increased in AML/MDS cases compared with controls (OR = 2.0), whereas the frequency of GSTT1 null genotype in AML/MDS cases was not statistically different from controls. [24] This type of study illustrates the hope that, in the future, molecular epidemiologists will be able to develop an individual's risk profile, including assessment of multiple biomarkers. The field has the near-term potential to have a significant impact on regulatory quantitative risk assessments, which may aid in the determination of allowable exposures. Molecular epidemiologic data may also aid in the identification of individuals who will most benefit from cancer prevention strategies.

Investigators who conduct molecular epidemiology studies use traditional designs, including case-control and cohort studies, with inclusion of one or more genetic markers to determine exposure associations with disease outcome. Scientists agree that chronic diseases, including cancer, likely result from gene-environment interactions. In fact, some researchers have said that “genetics is the loaded gun, and the environment pulls the trigger.” Many are concerned about the question of “nature versus nurture,” and how to evaluate the contribution of each component. A recent large study of twins, although statistically limited, concluded that environment plays a substantial role in causing sporadic cancers, but still requires genetic potential for cancer to occur. [25]

Methodologic challenges of epidemiologic studies (as described below), such as accurate measurement of disease and exposure, appropriate selection of study samples, reducing potential confounding, and optimizing precision of effect measures, also apply to studies in the rapidly growing and promising field of molecular epidemiology. A serious concern lies with assuring an adequate sample size for study. Often, the prevalence of a genetic polymorphism or other biomarker is either quite low or quite high. Hence, the number of cases required to detect an association tends to be very large. Because childhood cancers are rare, it is often necessary to combine data from several studies to obtain adequate statistical power to draw meaningful conclusions. All of these issues speak to the need for investigators to exercise caution when interpreting their study data and the implications of their results. [26]

## BIAS AND CAUSAL INFERENCE

All human studies are susceptible to bias of varying degrees (i.e., producing inaccurate measures of the effect of a treatment or exposure on disease). An important goal of any study is to make every effort feasible to minimize the effect of bias.

Three general types of bias can occur:

1. *Selection bias*, when subjects who are sampled, recruited, enrolled, and complete the study are unrepresentative, in that they inaccurately reflect the exposure-disease relation in the target population
2. *Information (misclassification) bias*, when information collected on exposure, treatment, disease, or other study factors is inaccurate or incomplete
3. *Confounding bias*, when an extraneous factor distorts (increases or decreases) the true magnitude of the exposure-disease association

### Confounding

Randomization in clinical trials, if enough people are in the study, greatly reduces the probability that an extraneous factor will cause bias in the results because such “nuisance” factors should be randomly and evenly distributed among treatment groups. Absent randomization, however, confounding is a threat to validity in observational studies. Confounding requires a variable to be associated with, or a marker for, the disease of interest and for it to occur at a differing frequency between the exposure (or treatment) groups. When these two conditions hold, the extraneous factor may bias the exposure-disease association. Few exogenous risk factors, however, have been identified in the etiology of childhood cancer, and those few represent fairly weak associations. Thus, confounding bias has not been shown empirically to be of major concern in epidemiologic research of childhood cancer, although this possibility cannot be ruled out. Partly because of the implausibility of a biologic connection between EMF and cancer, for instance, some scientists hypothesized that the associations found between power lines and childhood leukemia and brain cancer in early EMF studies [27,28] were due to confounding by unidentified etiologic agents. [29] A recent methodologic study that carefully examined that possibility found little support for the theory. [30]

Statistical methods to control (adjust for, or correct) confounding, such as pooled stratified analysis or multivariate regression analysis, are at hand, but effective only if data on the potentially confounding variables are collected and accurate. Thus, for statistical analysis, observational studies often collect data on many factors not directly related to the cause-effect relation being investigated. Design strategies can also minimize or eliminate confounding. A study of asbestos exposure and lung cancer, for example, could avoid confounding from smoking status by recruiting only non-smokers.

### Information Bias

The most important threat to the validity of epidemiologic research of childhood cancer is inaccurate or incomplete information on study participants' exposure relevant to etiology. It is usually impossible, especially in retrospective studies, to directly measure exposure dose and duration during a time thought biologically relevant to cancer initiation or progression. As such, indirect or surrogate measures of exposure are used in lieu of direct measures. Indirect exposure tools include, for instance, self-reported recall of diet, smoking, and alcohol consumption during pregnancy; 24-hour food intake diaries; parental occupational job titles; recall of household pesticide use or inventory of household pesticide products; power line configurations, personal dosimeters or 24-hour measurements of EMF levels in the child's bedroom; pharmacy records among those in self-contained health maintenance organization plans; census tract information; urinary cotinine levels for smoking intake; and medical records.

These proxy measures may usefully approximate real exposure, but provide only imprecise information on dose, duration, and exposure time period. When exposure measures are equally inaccurate between study groups (non-differential error), as is often the situation, the cause-effect relation may be attenuated or completely obscured. Non-differential misclassification of exposure has no doubt been one reason why few environmental agents are known risks for childhood cancer occurrence.

Differential information bias occurs when accuracy and completeness of exposure information differs between comparison groups. Recall bias in case-control studies, for example, can occur if mothers of children with brain cancer (cases) are more motivated than mothers of healthy children (controls) to recall accurately their history of using household pesticides. This may happen because case mothers want to discover the cause of their children's disease. The control mothers may have hazier memories, and their incomplete or inaccurate recall can lead to underestimates of exposure frequency in the control group, and thus cause exaggeration of the strength of the association between disease and exposure. From a practical standpoint, however, recall bias may be more theoretical than factual. [31] One method sometimes advocated to minimize recall bias is to choose a control group of children with a chronic disease, rather than disease-free. Control mothers might then have equal incentive to recall exposure accurately and completely. Using this approach, one must be sure that the control group's disease is not causally related to the exposure under evaluation, or the resultant risk estimate will be biased as to whether the exposure is causally related to the childhood cancer in question.

### Selection Bias

Because all human studies include some element of sampling from larger (target) populations and require recruitment from the sample identified, selection bias is a potential source of error. Selection bias may occur when exposure or disease frequency among those in the study is unrepresentative of the target population. Case-control studies are susceptible because it is difficult to identify and recruit controls who provide an accurate accounting of baseline exposure frequency in the population that gave rise to the cases. For instance, selection bias is suspected in the apparent association of some childhood cancer–EMF studies. [30,32] If low-income

persons are proportionately less likely to participate as controls than higher-income persons, and low-income persons live in areas with proportionately more high current power lines, baseline exposure (high EMF) will be underestimated. Unlike controls, if case participation is independent of power line status, the odds of exposure among cases will appear higher than that of controls, resulting in a positive association when none really exists. Cohort studies and randomized trials, on the other hand, are susceptible to selection bias from attrition. If participants lost to the study during the follow-up period represent a different outcome experience than those who remain in the study to completion, the final results may be biased. For this reason, great effort must be expended in prospective studies to assure the most complete follow-up possible of study subjects.

### Causal Inference

Epidemiologic studies strive to provide the most accurate and precise risk estimate of an exposure-disease association. Concerns about potential bias of effect measures, however, contribute to the critical approach using inference and judgment to evaluate exposure-disease causal relations. Criteria commonly used to evaluate study results and to help guide judgments on the likelihood that an association indeed is causal and not merely statistical, include

1. *Strength of the exposure-disease association*. Large relative risks are less likely than small relative risks to result from chance or uncontrolled confounding (although this does not preclude other sources of error).
2. *Temporal relation between exposure and disease onset*. Studies are stronger when they can establish that the exposure appropriately preceded the biologic onset of disease.
3. *Biologic coherence*. When a plausible biologic mechanism or when experimental evidence from animal studies, or both, supports the hypothesized relation, there is greater confidence in the observed relation.
4. *Dose response gradient*. If exposure intensity or duration is associated with increased disease frequency when it is hypothesized that such a dose gradient should exist, the results appear more coherent and believable.
5. *Consistency* of results within and across studies. If multiple sources of the same exposure type show similar effects, or if multiple studies using different target populations and study designs show consistent results, or both, there is greater evidence to favor a true relation.

These concepts, which are widely applied, were originally derived from two papers by Sir Austin Bradford Hill and recently reprinted in a monograph on philosophy and epidemiologic reasoning in causal inference. [33]

## STATISTICAL MEASURES IN EPIDEMIOLOGY

Epidemiologic analyses generally focus on estimating effect measures, the strength (magnitude) of an exposure-disease association, rather than statistical hypothesis testing using a *p* value.[2] *p* Values provide a measure of probability for observing the study results, or results more extreme than those observed, if indeed there is no true association. No direct information from *p* values is given, however, on the strength, direction, or precision of an effect measure. Nor do *p* values supply information on the extent to which an association (or lack of an association) can be explained by confounding or other bias.

Effect measures for dichotomous outcomes, such as disease occurrence versus no disease, are often estimated using one of several ratio measures of relative risk.[2,34] In a cohort study, in which disease rates can be directly calculated, the ratio of the incidence rate of leukemia among those exposed to an agent can be compared with the rate of leukemia among those not so exposed. The ratio is 1:1 if the rates are the same in the two comparison groups, a relative risk of 1.0, suggesting no association between exposure and disease. If the exposed group has a higher rate than the unexposed group, the ratio will be larger than 1, suggesting an excess risk due to exposure. If the rate is lower in the exposed compared with the unexposed groups, the ratio will be less than 1, suggesting a protective effect from exposure. The further the effect measure is away from the “null” value of 1.0 in either direction, the stronger the association. Notice that a relative risk of 2.0 (double the risk compared with the reference group) is equivalent in strength to a relative risk of 0.5 (one-half the risk of the reference group). Rates of disease cannot be calculated directly in case-control studies. Alternatively, exposure frequencies are compared between diseased groups and non-diseased groups. The resultant OR is an effect measure on a ratio scale and, as mentioned previously, functionally equivalent to a relative risk. Other types of ratio-based relative risks are rate ratios, hazard ratios, standardized mortality ratios, standardized incidence ratios, and proportional mortality ratios. Confidence intervals are used to measure the precision of an effect measure. Like *p* values, confidence intervals are functions of the variability of the data and the size of the sample. Roughly speaking, a confidence interval provides a likely range in which the true effect measure lies within some level of confidence (often calculated as 95% confidence intervals).

Relative risks are important to help judge whether an association is causal and to estimate the degree to which risk of disease is increased (or decreased) by exposure. Relative risks, however, do not measure the “absolute” risk from exposure. In other words, a relative risk does not measure the number of excess cancers that are likely caused by an exposure. Attributable risk measures provide estimates of the actual rate (or number, or percentage) of cases “due to” exposure, assuming there is a causal relation.[34] Thus, attributable risks indicate the proportion of the disease preventable, if the exposure were removed from the population at risk. Assume for the sake of argument, for example, that living within 50 ft of a high-current power line increases a child's risk of ALL by a factor of 2. The annual rate of ALL in the United States is approximately 34 per million children younger than age 15 years. [35] If 10% of children in the United States lived near high-current power lines, the percentage of childhood ALL cases that could be attributed to the power lines would be 9%. This attributable risk of 9% (sometimes called an *etiologic fraction*) translates to an excess of three ALL cases per million children per year, which is the leukemia rate that hypothetically would be prevented if all children lived away from high-current power lines. Even very large relative risks may explain little of the total disease incidence within a population. Children with Down syndrome have an estimated 20-fold excess risk of ALL,[36] but because the prevalence of Down syndrome is only approximately 1.3 per 1,000 live births, the percentage of ALL in children that can be attributed to Down syndrome is only approximately 2.5%.

## RISK FACTORS FOR CHILDHOOD CANCER OCCURRENCE

Environmental risk factors for adult cancer generally involve long latency periods from exposure commencement to clinical onset of disease. Cigarette smoking illustrates this point: Smoking usually starts during adolescence, but associated malignancies do not become apparent until many decades after smoking is initiated. The genetic processes that go awry and lead to childhood cancer are likely different from that of adult malignancies; at the least, the carcinogenic process in children is much shorter in time. Infancy, when embryonal neoplasms such as neuroblastoma predominate, is the age when cancer incidence rates are highest during childhood.[37] It is reasonable to surmise, therefore, that many childhood cancers result from aberrations in early developmental processes.

To our dismay from a prevention standpoint, the current evidence to support a major etiologic role for environmental or other exogenous factors in childhood cancer is minimal. A comprehensive review of epidemiologic studies of childhood cancer is available elsewhere [31] and will not be reproduced here. The major types of childhood cancer and the few risk factors that are reasonably well documented are shown in Table 2-1. Many other factors are suspected to increase or decrease risk, but are not well established. Even the known risk factors shown in the table explain only a small proportion of childhood cancer cases.

**TABLE 2-1. KNOWN RISK FACTORS FOR SELECTED CHILDHOOD CANCERS**

## SUMMARY

Although knowledge about childhood cancer continues to increase, there is much work to be accomplished before reliable preventative measures can be recommended. In this brief overview, we have discussed the essentials of epidemiologic research approaches in childhood cancer, the role epidemiology plays in understanding the public health impact of childhood cancer, and the ongoing efforts to improve knowledge on the causes of these diseases and the consequences to the children who experience them.

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# 3

# CHILDHOOD CANCER AND HEREDITY

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## INTRODUCTION

A question often arises in the minds of parents when their child is newly diagnosed with cancer: “Did this happen because of something I did or passed on to my child?” or “What are the chances that my other children will develop cancer?” In this chapter, we outline the scientific and clinical evidence that is available to answer these questions with regard to genetic susceptibility. Overall, the percentage of childhood cancers that are caused by a clearly inherited predisposition is low. That percentage varies with individual tumor types and is a composite of several different genetic factors. Over the last 10 years, the genes that are mutated in many of these syndromes have been identified, providing the opportunity for genetic testing. After reviewing these syndromes, we also discuss the special issues to be considered in genetic testing for the pediatric oncology patient.

## INHERITED PREDISPOSITION TO PEDIATRIC CANCERS

Overwhelming evidence demonstrates that cancer is the result of multiple mutations in the DNA of the tumor cell. Many of these somatic alterations are discussed in Chapter 4 and in the disease-specific chapters. In contrast to the predominance of somatic mutations, the proportion of pediatric cancers that have a clearly hereditary component is small. *Hereditary* in this case implies a genetic alteration that has been passed on to the child from a parent or that was a new constitutional mutation that occurred in the oocyte or sperm before fertilization. A child therefore can have a hereditary predisposition to cancer with a negative family history because of a constitutional chromosome disorder such as Down syndrome (DS) or a de novo mutation in a cancer predisposing gene such as *Rb*.

Estimates of the hereditary predisposition for an individual cancer are based on epidemiologic studies of the number of familial cases, studies of associated syndromes, and molecular studies if the particular gene involved in a tumor type is known. The percentage of cases due to hereditary factors varies widely among tumor types, as illustrated in Table 3-1, with adrenocortical carcinoma, optic glioma, and retinoblastoma (RB) having the highest inherited fractions and many other tumor types falling in the range of 1% to 10%.[1] For example, a survey of 900 leukemia patients from Australia revealed that 72 had a single first-degree relative with leukemia (twice that expected).[2] However, only a single proband had multiple affected relatives, suggesting that single-gene inheritance that predisposes the carrier to a high risk for leukemia is rare.[3] A study using an alternative approach examined 16,564 cases of childhood cancer[1] and found that 2.6% of children with leukemia had an associated genetic condition, including DS and neurofibromatosis (NF).

| Tumor type | Hereditary component (%)[a] |
|---|---|
| Adrenocortical carcinoma[117,118] | 50–80 |
| Optic gliomas[43] | 45 |
| Retinoblastoma[188] | 40 |
| Pheochromocytoma[267] | 25 |
| Wilms' tumor[31,43] | 3–5 |
| Central nervous system neoplasms[1,345,346] | <1–3[b] |
| Leukemia[1,2] | 2.5–5.0 |

[a]These percentages are approximations from large population studies and may include familial cases and associated syndromes such as Down syndrome.
[b]Studies of pediatric brain tumors vary considerably in detection of a hereditary fraction.

**TABLE 3-1. HEREDITARY COMPONENT OF SEVERAL PEDIATRIC MALIGNANCIES**

Geneticists categorize disorders by the mechanism of inheritance: constitutional chromosomal abnormality; mendelian autosomal dominant, recessive, or X-linked patterns; and nonmendelian inheritance. The latter category includes inherited disorders that are caused by mutations in multiple genes or mitochondrial DNA or caused by mutations affecting imprinted genes. For any tumor type, the overall inherited fraction is the sum of several different genetic mechanisms. For example, an increased risk of Wilms' tumor (WT) is associated with multiple genetic syndromes ( Table 3-2), including a chromosomal deletion syndrome, an autosomal dominant disorder, and a syndrome resulting from disruption of imprinting.

| Disorders predisposing to Wilms' tumor | Mechanism of inheritance |
|---|---|
| WAGR | Interstitial deletion of chromosome 11p13, including *WT1* gene |
| Denys-Drash syndrome | Autosomal dominant—missense mutation in *WT1* |
| Autosomal dominant Wilms' tumor | Autosomal dominant—genes unknown |
| Hemihypertrophy and Beckwith-Wiedemann syndrome | Maternal deletion, uniparental disomy, or disordered imprinting of 11p15 |

WAGR, syndrome including Wilms' tumor, aniridia, genital abnormalities, and mental retardation.

**TABLE 3-2. MULTIPLE GENETIC MECHANISMS THAT INCREASE THE RISK OF DEVELOPING WILMS' TUMOR**

In the following sections, we describe the major types of hereditary disorders. In addition to defined genetic disorders, there may be rare families that show clustering of a particular tumor type. Presumably, these families represent mutations in genes that predispose the members to specific cancers. For example, neuroblastoma has been reported to cluster in very rare families,[4] and current research is focused on identifying the gene mutated in these families that predispose them to this specific cancer.

## CONSTITUTIONAL CHROMOSOMAL ABNORMALITIES

Children with constitutional chromosomal abnormalities present with defined clinical phenotypes that can include dysmorphic features, congenital abnormalities, growth failure, and developmental delay. Most result from errors that occurred during oogenesis or spermatogenesis, with both parents having a normal karyotype. Rarely, these disorders can result when a parent is a carrier for a balanced translocation. Constitutional chromosomal abnormalities are the result of an abnormal number (i.e., aneuploidy) or structural rearrangements of the normal 46 chromosomes (i.e., 22 pairs of autosomes and the sex chromosome pair). Because of the diagnosis in childhood of these syndromes by clinical phenotype and karyotype analysis, an increased association of these disorders with malignancy was recognized early.

### Down Syndrome

One of the most striking predispositions to cancer caused by a constitutional chromosome abnormality is the increased risk of leukemia in children who have trisomy 21.[5,6] An analysis of the Danish population results in an estimated cumulative risk for developing leukemia of 2.1% by 5 years and 2.7% by 30 years. [7] Other studies yield a slightly lower risk of leukemia of 1% by age 10.[6] This represents at least a 20-fold increase compared with the risk for the general population. Trisomy 21 is also a common finding in the karyotype of leukemia cells from patients without DS.[8,9] Thus, presence of an extra chromosome 21 appears to be leukemogenic and may be acquired in the germline or somatically. The specific locus on chromosome 21 responsible for this increased risk of leukemia is unknown. The recent publication of the complete sequence of human chromosome 21 should facilitate this area of research. [10]

In children with DS, the ratio of lymphoid to myeloid leukemia is shifted to 60% lymphoid and 40% myeloid from the ratio in the general population of 80% lymphoid and 20% myeloid.[7] This shift is principally due to the increased incidence of myeloid leukemias in children younger than 2 years of age. An analysis of data from several large Pediatric Oncology Group protocols compared the phenotype at presentation and result of therapy for acute lymphoblastic leukemia in children with and without DS.[11] Overall, the children with DS presented with more favorable leukemic subtypes, as measured by a decrease in the chromosomal translocations that are associated with a poor prognosis. For example, there were no children with the t(9;22), t(1;19), or t(4;11) translocation in the DS group, compared with an expected frequency of 10% to 13% in the non-DS (NDS) population. However, the DS children experienced more toxic effects from the chemotherapy, and their overall outcome therefore was not better than the NDS patients. Analysis of children in the United Kingdom with DS and leukemia treated between 1980 and 1994 also found a decreased survival (57% versus 75% 5-year disease-free survival) for the children with DS. [12]

Most striking is the distribution of types of leukemia among the DS children who develop acute myeloid leukemia (AML). [6] Approximately 30% of DS children with AML develop acute megakaryocytic leukemia (AMKL or M7). This results in an almost 400-fold excess of this particular malignancy in the DS children compared with NDS children. Biologically, children with DS and AMKL also show a different phenotype. In one analysis of 116 children with AMKL, 16 of the NDS children had the characteristic t(1;22)(p13;q13) translocation, compared with none of the DS children. [9] NDS children tended to present in early infancy and to have significant hepatomegaly, but the DS children, on average, presented at 23 months, and a high proportion had myelofibrosis.

Another unusual phenotype of children with DS is the development in infancy of a transient myeloid proliferative syndrome that can appear similar to leukemia but that is self-limited.[13,14] However, 25% of DS children with this syndrome eventually develop frank AML. Children who are mosaic for trisomy 21 in their blood and bone marrow have also developed transient myeloproliferative disorder and subsequent leukemia. [15,16] Similarly, children with DS have a higher rate of myelodysplasic syndromes, which are characterized by thrombocytopenia, abnormal megakaryocytopoiesis, and an abnormal karyotype, most commonly trisomy 8.[5] DS children with transient myeloproliferative disorder, myelodysplasic syndrome, and AMKL also all appear to have a propensity for complete remission. [5] The molecular bases for these differences remain to be determined and may provide insights into the genes responsible for spontaneous remission.

Despite the well-documented increase in the risk of leukemia in children with DS, a study based on exhaustive analysis of the Danish population found no increased risk of solid tumors in children or adults with DS. In particular, cases of breast cancer were significantly less than expected in DS adults compared with an age-matched population.[7]

### Sex Chromosome Abnormalities

Sex chromosome abnormalities comprise a large group of disorders that result from numeric and structural problems with the X and Y chromosomes. The overall incidence of sex chromosome abnormalities is high, with Klinefelter syndrome and Turner syndrome each affecting approximately 1 in 2,000 individuals. The diagnosis of these disorders, unlike DS, is often not made until late adolescence or young adulthood, when problems with the transition through puberty and fertility become apparent. However, children with these disorders are at increased risk for certain malignancies during childhood, arguing for earlier diagnosis.

#### *Y Chromosome*

Any phenotypic female with part or all of a Y chromosome is at risk for development of gonadoblastoma in her streak gonads. [17] The risk can be as high as 25% for individuals in the late second or third decade. Children with this problem include girls with androgen resistance syndromes (i.e., testicular feminization) who have a normal 46XY karyotype, children with gonadal dysgenesis, and girls with Turner syndrome and a mosaic 45X,46XY karyotype. Mosaicism results from an individual with several different populations of cells presumably due to a 46XY zygote losing a Y chromosome in an early mitosis. Approximately 25% of girls with Turner syndrome have some evidence for mosaicism.[18] The *TSPY* gene on the Y chromosome has been implicated as the gene responsible for gonadoblastoma in these conditions (reviewed by Lau[19]).

Phenotypic girls with a Y chromosome component should have prophylactic surgery to remove their gonads. In most circumstances, these gonads are nonfunctional, and removal does not affect the girls medically. However, the discovery of a sex chromosome karyotype that is not consistent with their phenotypic sex can be devastating for patients and their parents and should be carefully handled by a medical team familiar with these disorders. [20]

#### *Klinefelter Syndrome*

The clinical phenotype of Klinefelter syndrome (47XXY) is variable and includes tall stature, infertility, decreased secondary sex characteristics, and gynecomastia. Men with Klinefelter syndrome are often not diagnosed until adulthood, making epidemiologic studies of the increased risk of malignancy in childhood difficult. Nonetheless, some studies suggest an increased risk of dysgerminomas[21] and extra-gonadal germ cell tumors.[22] Men with Klinefelter syndrome have an increased risk of breast cancer[23] that does not appear to correlate with gynecomastia.[24,25] There is controversial evidence for an increased risk of leukemia in men with Klinefelter syndrome, and one large cytogenetic study of men with leukemia demonstrated no increased incidence of 47XXY. [26]

## STRUCTURAL CHROMOSOMAL ABNORMALITIES

### Detection and Impact

As cytogenetic techniques were improved in the 1960s and 1970s, it became clear that many of the complex dysmorphic syndromes were the result of large deletions that could be detected cytogenetically. During the 1980s to 1990s, the development of specific molecular probes and techniques to assay deletions by Southern blot analysis and fluorescent *in situ* hybridization (FISH) permitted further progress in mapping and identifying the underlying cause of these syndromes. Interstitial deletions can result in the loss of several contiguous genes, and the varied phenotype of a particular disorder may result from the loss of these often unrelated neighboring genes. The size of the deletion impacts how many of these genes are lost and how many features of a syndrome the child may manifest. Chromosomal deletions may be de novo events or inherited from either parent. Although these disorders are the results of deletions, the syndromes overlap with autosomal dominant disorders that are the result of smaller mutations affecting a single gene. For example, RB, which can be inherited as an autosomal dominant disorder due

to point mutations in the *Rb* gene, is also associated with a cytogenetically visible deletion in a small percentage of cases. [27] The cytogenetically visible deletions have led to early localization and cloning of these genes in several cases.

### Wilms' Tumor, Aniridia, Genital Abnormalities, and Mental Retardation

The WAGR syndrome is named for the components of the disorder: Wilms tumor, niridia, genital abnormalities, and mental retardation. Riccardi [28] and Francke[29] demonstrated that several children with AGR also had WT, and three of these children had cytogenetically detectable deletions at 11p13. Surveys of children with WT in the United Kingdom[30] and France[31] revealed that 3% and 1%, respectively, of children with WT had aniridia. The children with both WT and aniridia demonstrated mental retardation and genital abnormalities, including hypospadias.

Work from the Housman laboratory defined the minimally deleted region in WAGR and specified the areas responsible for WT, aniridia, and mental retardation. [32,33] This work led to the successful cloning of the gene, *WT1*, responsible for the WT phenotype.[34] *WT1* encodes a zinc finger transcription factor (reviewed by Little [35]). All or part of *WT1* is deleted in children with WAGR and WT. [36] In contrast, point mutations in *WT1* are found in children with the Denys-Drash syndrome, a disorder characterized by severe urogenital abnormalities and WT. [36,37] This is an example in which total loss of a gene product through deletion results in a less severe disease than production of a mutant protein due to a missense mutation. The gene responsible for the aniridia phenotype has also been cloned and is deleted in children with WAGR[38] and sporadic aniridia.[39]

Surprisingly, somatic mutations in the *WT1* gene in sporadic WT are found in only 10% of cases. [35] Also, families with a pattern of cancer consistent with autosomal dominant WT do not have mutations in the *WT1* gene.[40] Linkage studies in familial Wilms' kindreds suggest that there may be more then one locus involved. [41] None of these genes has been identified to date.

Any child with aniridia should have cytogenetic studies, including FISH analysis for deletion of the *WT1* gene, and be carefully screened for the development of WT. Screening of children with WAGR or Denys-Drash syndrome is often carried out by abdominal ultrasound examinations every 4 months until the age of 5 years, with decreasing frequency of examinations at later ages.[42] The recommendation for serial ultrasound scans is controversial and is based on small numbers. The National Wilms' Tumor Study found more stage 1 tumors in children who had been screened. [42] However, the Childhood Cancer Research Group in Oxford found that eight children who had their WTs diagnosed by ultrasound screening did not have more favorable outcomes than those in the group that was not screened. [43] Parents should be counseled to bring the child in for evaluation if they suspect any change in abdominal girth or feel a mass, regardless of whether ultrasound screening is performed.[43,44]

A long-term analysis of children with WT and either Denys-Drash or WAGR found a 62% and 38% rate of renal failure 20 years after the diagnosis of WT. [45] Therefore, children with WT and one of these syndromes require long-term follow-up for evidence of declining renal function.

## OVERGROWTH DISORDERS AND IMPRINTING ERRORS

### Beckwith-Weidemann Syndrome

Not surprisingly, there has long been a recognized relation between disorders of increased growth and predisposition to cancer. Two related syndromes in particular, Beckwith-Wiedemann syndrome (BWS) and hemihypertrophy (HH), are linked to a significantly increased risk of developing abdominal tumors, including WT and hepatoblastoma.[31,46] Of 183 children in the BWS Registry, 13 had developed a tumor by age 4. [47] BWS is characterized by excessive intrauterine and postnatal growth, organomegaly, macroglossia, and unusual linear ear creases.[46] The organomegaly can lead to omphalocele and umbilical hernias. HH in a child is defined as asymmetric growth due to overgrowth of one side relative to the other. It can be limited to a limb or the face or include the whole side. HH can be a feature of BWS or an isolated finding. For children with HH, the risk of WT is approximately 3%. [42] Several studies have demonstrated that children with both BWS and HH have a higher risk of WT than children with either condition alone. [48,49] More recently, studies have also demonstrated that nephromegaly is associated with an increased risk of WT in children with BWS.[50]

The genetic basis of BWS and HH is complex (reviewed by Weksberg [51]). Some families have an apparent autosomal dominant pattern that maps to 11p15.[52,53] In these families, BWS is more likely to be inherited from mothers than fathers. [53,54] There are also children with cytogenetically visible rearrangements in the region of 11p15 that are the result of duplications of the paternal chromosome 11p (reviewed by Slavotinek [55]). Moreover, there are children with apparently sporadic BWS. The mechanisms behind these unusual genetics may be clarified by understanding genetic imprinting. *Imprinting* refers to the fact that certain genes are expressed differently, depending on whether they were inherited from the maternal or paternal chromosome (reviewed by Tycko [56]). This form of inheritance was not predicted by Mendel's laws and can result in unusual pedigrees (e.g., unaffected sisters that can pass on a mutation in an imprinted gene to their children, resulting in affected cousins). BWS may result from cytogenetically normal children who inherit two copies of a paternal chromosome 11 and no maternal copy, termed *uniparental disomy*. Children with BWS and HH may show uniparental disomy of chromosome 11.[57]

There has been significant effort made to identify which imprinted gene is disrupted in BWS. Initial studies focused on the insulin-like growth factor-2 gene (*IGF-2*) in 11p15 as causative in BWS (reviewed by Reik[58]). There are now at least three genes that are imprinted in this locus that have been implicated in BWS. [59,60] *IGF-2* is a maternally imprinted gene (expressed from the paternal copy) involved in growth control. Paternally imprinted genes that show loss of imprinting in BWS include a cell-cycle inhibitory gene (*KIP2*),[61] *H19*, and *LIP1*.[62] Rare patients with BWS have been shown to carry a mutation in the *KIP2* gene.[63,64] Studies of mouse models demonstrate that alterations in both *IGF-2* and *KIP2* can contribute to the BWS clinical phenotype. [65]

Overall, given the increased risk of WT in these conditions, screening for WT by regular serial ultrasound examinations is recommended for children with BWS, HH, or both (see the preceding WAGR section for details about screening). Children with BWS screened for WT were much less likely to present with advanced disease then those who were not screened (0 of 12 versus 25 of 59).[66] Screening until age 8 detects the majority of children with BWS who will develop WT.

### Paraganglioma

Paragangliomata represent an unusual constellation of tumors variously termed *glomus tumors*, *chemodectomas*, and *carotid body tumors*. They essentially comprise a family of neoplasms that arise from paraganglia tissues—chemoreceptor organs distributed throughout the body. Approximately 20% of paragangliomas are familial in their presentation, and in an affected individual, they may occur either unilaterally or bilaterally. [67] The mode of inheritance is thought to be autosomal dominant with incomplete penetrance and variable expression, demonstrating both intra- and inter-familial variability. Maternal imprinting has also been suggested by pedigree analysis, although its molecular mechanism is unknown. At least three genes have been linked to hereditary paraganglioma, with the *PGL1* gene on chromosome 11q23 having been recently cloned.[68] *PGL2* is localized to chromosome 11q13, whereas *PGL3* has not yet been localized, nor do these families exhibit a maternal imprinting pattern.[69,70] Like other genes associated with familial predisposition to cancer, *PGL1* behaves as a tumor suppressor at the cellular level (as described below); it encodes a mitochondrial respiratory chain protein termed *cybS* (cytochrome b small subunit), which is thought to play a role in the $O_2$-sensing system of paraganglionic tissue.[68] Its loss may lead to chronic hypoxic stimulation and cellular proliferation. As with the hereditary overgrowth syndromes, BWS, and others, the complex mechanisms behind maternal imprinting and the unusual phenotypic expression patterns indicate the strong need to ensure that complete and accurate multigenerational family pedigrees are obtained on children with cancer to facilitate effective genetic counseling.

## MENDELIAN INHERITANCE OF A PREDISPOSITION TO CANCER

There are many single-gene disorders that result in an increased cancer risk and are inherited in a fashion consistent with single-gene disorders. In the following sections, we discuss a sampling of the disorders that are relevant to pediatric oncology and that have provided insight into cancer genetics.

### Autosomal Dominant Disorders

Autosomal dominant syndromes comprise the majority of families with single-gene disorders that convey an increased risk of cancer. The features of autosomal dominant inheritance are described in Table 3-3. The disorders are transmitted from the father or mother to a son or daughter, in contrast to X-linked disorders. Often,

there is a multigenerational pattern, and similar to other autosomal dominant conditions, there is variable expression of the disorder within a family, with “skipped” generations (at the phenotypic level) because of incomplete penetrance. *Penetrance* is defined as the probability that a person inheriting the mutation will have the disease. This results in individuals with the same genetic mutation who have a variable phenotype.

| |
|---|
| Multiple generations affected with cancer |
| Transmission through mothers and fathers |
| Earlier age of onset of cancer compared with sporadic cases |
| Increased incidence of multiple and bilateral tumors |
| A clustering of increased risk of one or a few tumor types in the family |
| Variable penetrance can lead to carriers not developing cancer |

**TABLE 3-3. FEATURES OF AN AUTOSOMAL DOMINANT CANCER FAMILY SYNDROME**

***Retinoblastoma***

Much of our knowledge of autosomal dominant cancer families was gained from the study of RB. In a series of landmark papers in the early 1970s, Knudson and Strong performed statistical analysis of children with RB and other pediatric malignancies. [71,72,73 and 74] In 1971, Knudson's hypothesis was that bilateral RB represented the familial form, and those patients had already acquired one “hit” or mutation. [71] The best model consistent with his data indicated that the bilateral form required only one additional hit but that the unilateral form required two hits. Given the epidemiologic nature of the study, the physical basis of the second hit was unknown, but the first was presumably a genetic mutation due to its inherited nature. Before this work, in 1968, Nicholls [75] had proposed that the skin lesions in NF type 1 (NF1) represented two mutational events in the same gene, with the first mutation being inherited and the second mutation occurring somatically. RB may be exceptional in requiring so few mutational events for tumors to develop, because many more than two mutations are required for most adult tumors. [76]

The clinical features of syndromes that follow the two-hit hypothesis are shown in Table 3-3. The most striking features are those initially observed by Knudson: Familial forms of RB present earlier and with a greatly increased percentage of bilateral and multiple primary tumors. Importantly, some patients (approximately 15%) with unilateral disease carry a constitutional mutation. An even milder form of retinal tumor, retinoma, that spontaneously regresses can be seen in apparently unaffected adults. Approximately 10% of people with a germline mutation in RB do not develop RB (i.e., incomplete penetrance). [77] The penetrance varies among families, however, with some mutations characterized by a higher likelihood of developing unilateral disease, termed *attenuated RB*. [78,79]

An additional phenotype common to the autosomal dominant conditions has been found for RB families by long-term follow-up of childhood survivors. Individuals carrying germline mutations in the *Rb* gene are also at increased risk for development of other primary tumors. In particular, there is an increased risk of osteosarcoma [74,80,81] and malignant melanoma. [81,82] Overall, there was a 26% mortality by age 40 from secondary neoplasms in children with bilateral RB. This finding was higher for children treated with radiation and substantially lower for unilateral cases. [83]

Based on the visible cytogenetic deletions, the gene mutated in RB was isolated. [84] Molecular studies allowed confirmation of Knudson's two-hit hypothesis. RB requires loss of both copies (i.e., two hits) of the *Rb* gene for a tumor to develop (Fig. 3-1; see Chapter 28). The normal function of the *Rb* gene product is to negatively regulate the cell division cycle. [85,86] The loss of this function, called *tumor suppression*, is consistent with loss of cell cycle control. In the familial form, a mutation in one *Rb* gene is inherited, and therefore all the cells in the body have only one normal allele. If during development that normal copy is mutated or lost, cell cycle control is disrupted and RB can develop. The most common mechanisms by which the second copy is lost are loss of the whole chromosome, large deletions, and gene conversion normally resulting in loss of heterozygosity for markers near the *Rb* locus. In the sporadic form, mutation or loss of both *Rb* genes must occur in the same somatic retinal cell for RB to develop.

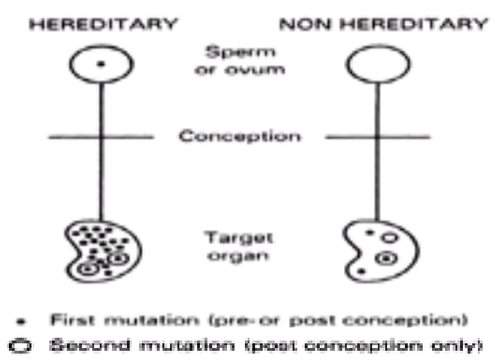


**FIGURE 3-1.** Knudson's two-mutation hypothesis. In all tumors, the same cell must undergo at least two mutations to become malignant, and the second mutation always occurs after conception. In sporadic, nonhereditary tumors (*right*), the first hit also occurs after conception. In hereditary tumors (*left*), the first mutation is in a germ cell, such that all body cells in the offspring have the first mutation. (From Miller RW. Genetics and familial predisposition. In: Calabresi P, Shein PS, Rosenberg SA, eds. Medical oncology: basic principles and clinical management of cancer. New York: Macmillan, 1985:130.)

Although all bilateral patients have been documented to carry constitutional mutations in the *Rb* gene, 80% have no family history of RB. This is due to the majority being the result of a de novo mutation in the *Rb* gene. In Table 3-4, the risk of having a second child with RB for parents of a child newly diagnosed with either unilateral or bilateral RB is given. [77] Surprisingly, for parents of a child with bilateral RB who have normal eye examinations, they retain a 6% risk to have a second affected child. This is because the de novo mutation may occur during the father's germline development and result in a variable percentage of the sperm carrying the mutation (germline mosaicism). A recent analysis of normal parents of 156 children with bilateral RB documented mutations in the father's germline in at least 10% of cases. Therefore, if genetic testing is not pursued, all siblings of children with bilateral RB should have ophthalmic surveillance beginning at birth.

| Clinical scenario | Recurrence risk (%) |
|---|---|
| Offspring of bilateral cases | 45 |
| Offspring of unilateral cases | 7.5 |
| Sibling of bilateral cases (parents unaffected) | 6 |
| Sibling of unilateral cases (parents unaffected) | 1 |
| Sibling of bilateral or unilateral cases (if either parent is affected) | 45 |

Adapted from Musarella MA, Gallie BL. A simplified scheme for genetic counseling in retinoblastoma. J Pediatr Ophthalmol Strabismus 1987;24:124–125.

**TABLE 3-4. EMPIRICAL RECURRENCE RISKS IN FAMILIES WITH RETINOBLASTOMA**

The discovery of the *Rb* gene[84] has allowed for molecular diagnostics to be offered to families.[87] Several different approaches are used to identify mutations.[88] Standard karyotype analysis coupled to FISH studies using RB probes may reveal deletions of the entire gene, but in fewer than 5% of germline mutation patients.[27] The presence of smaller deletions in a small number of cases also are detected by Southern blot analysis. The remaining mutations that are scattered throughout the gene can only be found by detailed sequence analysis, which, although commercially available, is expensive and labor-intensive.[89] The majority of these mutations results in a truncated protein or disrupt specific functional domains of the Rb protein.[88]

Because a patient with a negative family history and unilateral disease, has only a 15% a priori chance of having a germline mutation, discerning the difference between a pathologic missense mutation or benign change is difficult. For this reason, many molecular diagnostic laboratories first identify mutations in the *Rb* gene in a tumor specimen (for unilateral cases) and then determine if the mutation can be identified in constitutional DNA from the blood.[90] Testing of bilateral RB patients is done directly from a blood sample.

There are several clinical situations in which molecular diagnostic testing is useful. Unaffected parents of a child with bilateral disease often are concerned about their risk for having additional children with RB. The physician first looks for the mutation in the affected child and then studies both parents to ascertain whether they carry the mutation. If a parent is positive, he or she has close to a 45% recurrence risk. As mentioned, even if both parents are negative, they retain a 6% risk of having an affected child due to mosaicism. Siblings of the proband can be tested at birth, and only those positive siblings need surveillance for RB. The adult survivors of childhood RB can also use DNA testing for prenatal diagnosis or immediate postnatal diagnosis of their own children. Current recommendations for ophthalmic surveillance include examination in the first few days of life and then serial examinations every 4 months until 2 years of age (see Chapter 28). In contrast, if DNA diagnostics demonstrate that the child did not inherit the mutation found in the affected relative, the surveillance and anesthesia required for thorough examinations can be avoided, thereby decreasing costs and potential morbidity.[91] Testing of unilateral patients can be particularly informative for parents. If it can be documented that the child does not carry a constitutional *Rb* mutation then (a) the child is not at substantial risk for secondary malignancies, (b) radiation therapy is associated with less hazard, and (c) the parents and eventually the patient have a negligible recurrence risk of having another child with RB.

***Inherited p53 Mutations, the Li-Fraumeni Syndrome, and Its Variant Phenotypes***

In 1969, an inherited cancer predisposition syndrome was reported by Li and Fraumeni on the basis of characterization of four families in which at least two cases of sarcoma occurred in early life.[92,93] Other cancers noted at an increased frequency in these families included premenopausal breast cancer, leukemia, and other sarcomas. Based on prospective analysis of these and other families, these investigators subsequently defined the “classic” syndrome as a proband with sarcoma diagnosed younger than age 45 years, with a first-degree relative with any cancer younger than 45 years, plus another first or second-degree relative with either any cancer younger than 45 years or a sarcoma at any age.[94,95] In addition to sarcomas and premenopausal breast cancer, an excess of brain tumors, leukemias, and adrenocortical carcinomas were noted.[94] An example of a pedigree from a Li-Fraumeni syndrome (LFS) family is shown in Figure 3-2. As more families have been ascertained, the list of possible or probable component tumors has expanded to include gastric cancer, lymphoma, and possibly early onset lung cancer, choroid plexus carcinoma, and colorectal cancer.[96,97 and 98] Birch and colleagues described several families that did not conform to the criteria of the classic LFS that they termed *LFS-like* (LFS-L).[98] The LFS-L families were defined on the basis of a proband with any childhood cancer or sarcoma, brain tumor, or adrenocortical carcinoma diagnosed younger than 45 years of age with one first- or second-degree relative with a typical LFS cancer diagnosed at any age, plus a first- or second-degree relative in the same parental lineage with any cancer diagnosed younger than the age of 60 years. In addition to the wide spectrum of tumor types observed in LFS, Hisada and colleagues showed that gene carriers are at significant risk of developing multiple synchronous or metachronous non-therapy induced neoplasms.[99] In particular, the overall relative risk of occurrence of a second cancer was 5.3 (95% confidence interval = 2.8 to 7.8), with a cumulative probability of second cancer occurrence of 57%.

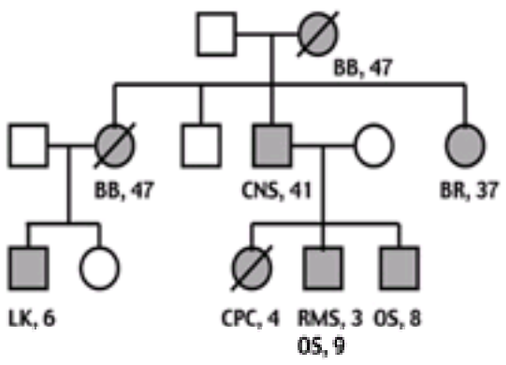


**FIGURE 3-2.** Pedigree of a family with Li-Fraumeni syndrome. Filled circles and squares represent affected members; circles with slashes represent deceased family members. Numbers represent age at diagnosis. BB, bilateral breast cancer; BR, unilateral breast cancer; CNS, brain tumor; CPC, choroid plexus carcinoma; LK, leukemia; OS, osteosarcoma; RMS, rhabdomyosarcoma.

Given the high mortality rate for affected members of LFS families, it was not possible to obtain DNA from extended pedigrees to carry out linkage analysis. In 1990, Malkin and colleagues took a candidate gene approach to determine the underlying genetic lesion in LFS.[100] Based on earlier observations that somatic mutations of the *p53* tumor suppressor gene were observed in more than 50% of sporadic human cancers,[101] and that p53 transgenic mice carrying mutant *p53* alleles developed a wide spectrum of malignancies,[102] these investigators elected to examine this gene in constitutional DNA of LFS kindreds. Heterozygous point mutations were initially detected in five of five families, however, numerous subsequent studies by these and other investigators have shown that only 60% to 80% of “classic” LFS families harbor detectable germline *p53* mutations,[103,104,105,106 and 107] whereas the majority of LFS-L families do not have detectable *p53* mutations in the coding regions of the gene.[107,108] Although a number of possible explanations, including mutations in promoter regions, have been proposed to explain the lack of *p53* alterations in all “classic” LFS families and a high proportion of LFS-L kindreds, none have been clearly documented experimentally.[109] Although it was originally observed that mutations in LFS occurred in a tight cluster within exon 7,[100,103] subsequent studies have confirmed that, in fact, mutations occur throughout the gene, though are primarily confined to highly conserved regions.

Several groups have examined the role of other tumor suppressor genes in these LFS, LFS-L families and individuals with the occurrence of multiple tumors. To date, these studies have been non-informative for germline alterations of *PTEN*, *p16INK4a*, and *p19Arl*. Other genes encoding proteins involved in p53-mediated cellular growth regulatory pathways, either effectors, targets, or binding partners of p53, have also been studied, but no germline alterations detected. Recently, an intriguing observation of heterozygous germline mutations in the checkpoint kinase *hCHK2* in one LFS family and one LFS-L family suggests an alternative mechanism for functional p53 inactivation in LFS.[110] This gene is the human homolog of the yeast *cds1* and *RAD53* G2 checkpoint kinases that are involved in preventing cellular entry into mitosis in response to DNA damage. Studies of murine embryonic stem cells deleted for *Chk2* confirm that the Chk2 kinase and p53 act together in the DNA damage response pathway, providing a molecular rationale for mutations in both genes being responsible for LFS.[111]

*p53*-Deficient mice were generated by Donehower and colleagues in 1992, and subsequently by other groups.[112,113 and 114] These mice have a striking propensity to develop a wide spectrum of cancer at extremely early age (younger than 9 months), with a relative prevalence of lymphomas. Interestingly, *p53*-heterozygous mice, harboring one wild-type and one mutant allele, also have a high incidence of cancer, although the tumors develop at a much slower rate.[115] Furthermore, in a pattern similar to the human LFS, these mice have a higher incidence of sarcoma development. Multiple primary tumors occur as well, again mimicking the human LFS phenotype. Although *p53* behaves as a classic tumor suppressor gene, less than 50% of tumors from *p53*-heterozygous mice and LFS patients have evidence of loss of heterozygosity.[115,116] It remains unclear in these patients how the retained wild-type p53 allele is functionally inactivated en route to malignant transformation of the

cell.

A number of studies have analyzed groups of patients with tumors characteristic of LFS, yet lacking characteristic family histories of cancer, for germline *p53* mutations. Such mutations have been identified in approximately 50% to 80% of children with adrenocortical carcinoma, [117,118] 10% of children with osteosarcoma,[119] and 10% of children with rhabdomyosarcoma.[120,121] The age of onset of tumors in the latter group of patients is strikingly lower (average age approximately 22 months) than in rhabdomyosarcoma patients with intact constitutional *p53*.[120] These observations suggest a possible difference in the biologic nature of malignant transformation of cells in which *p53* is altered as an early in contrast to a late event. One-third of children with sarcomas plus either multiple primary tumors or a family history of cancer have germline *p53* mutations. However, although breast cancer is a principal component of LFS, only 1% to 2% of women with familial, early-onset, or bilateral breast cancer harbor germline *p53* mutations.[122,123]

Presymptomatic molecular testing for germline *p53* mutations in members of Li-Fraumeni kindreds has been met with significant controversy. Because of the variable expressivity, the diverse tumor spectrum, and lack of clear clinical surveillance, preventive, or treatment recommendations, it is unclear how to manage the detection of a *p53* mutant carrier. Furthermore, the concept of predictive genetic testing of a child for a disease that may (or may not) occur in young adulthood poses significant challenges to the perception of the ethics of disclosure of genetic test results, in which the potential beneficiary of these results may wish to uphold the right to “not know.” In an attempt to address these issues, guidelines for testing have been established by both the American Society of Human Genetics and the American Society of Clinical Oncology. [124,125] These guidelines form a useful foundation on which to build practical testing parameters as better-defined genotype:phenotype correlations are generated.

After the publication of the statements of the American Society of Human Genetics and the American Society of Clinical Oncology, the Ethical, Legal, and Social Implications program sponsored by the National Center for Human Genome Research has created an extensive curriculum in Cancer Genetics and Cancer Predisposition Testing that discusses not only the principles of cancer genetics, but relates these to pediatric cancer syndromes as well as to cancer risk assessment, predictive testing, and Ethical, Legal, and Social Implications program concerns. The American Society of Clinical Oncology is making these curricula available to clinical oncologists. The recommendations outlined in these diverse “guidelines” can be applied to a number of cancer-predisposing gene testing programs. Although the interpretation of who should be tested remains a somewhat controversial point, certain common recommendations exist, including: the necessity that cancer risk counseling be part of the mission of clinical oncologists, the need for informed consent, formats for regulation of genetic testing, and continued efforts to address research issues. Specific concerns related to predictive genetic testing for children at risk continue to be raised, particularly when clinical screening protocols of those found to be mutation-positive are not defined.

### *Multiple Endocrine Neoplasia: Inheritance of a Mutation in an Oncogene*

The multiple endocrine neoplasia (MEN) disorders represent at least three different diseases, which are all autosomal dominant cancer family syndromes that affect different endocrine organs. MEN type 1 (MEN1) is characterized by parathyroid, pancreatic islet cell, and pituitary gland involvement (reviewed by Pang and Thakker[126]). Parathyroid involvement is found most frequently, and individuals from MEN1 families can also have their disease complicated by Zollinger-Ellison syndrome. The gene for MEN1 was mapped to human chromosome 11[127] and the gene, *MEN1,* identified.[128] By age 15 years, 28% of mutation carriers have either biochemical or clinical evidence for disease. [129]

Both MEN2A and MEN2B syndromes present in the pediatric period. MEN2A is associated with medullary thyroid carcinoma (MTC), parathyroid adenomas, and pheochromocytomas. MEN2B is a related disorder, but with the onset of tumors in infancy, ganglioneuromas of the gastrointestinal tract, and skeletal abnormalities. Additional families appear to show autosomal dominant MTC without the other features of MEN2A. Because of the life-threatening potential of MTC, treatment for MEN2 is prophylactic thyroidectomy in childhood.[130]

Before identification of the genes responsible for MEN2A and MEN2B, complex biochemical screening regimens were developed to detect patients requiring surgery early in the disease. These screening tests were based on assays of increases in the release of calcitonin in response to a pentagastrin challenge (discussed by Ponder[131]). With the discovery of the molecular basis of MEN2A and MEN2B, these biochemical assays have been replaced by genetic testing.

The gene for MEN2A was initially mapped to a small region on human chromosome 10q11.2.[132,133] The proto-oncogene *RET*, a receptor tyrosine kinase gene, mapped in this region and was known to be translocated in thyroid papillary carcinoma. [134] Analysis of constitutional DNA from multiple MEN2A families revealed a set of highly consistent mutations in the *RET* gene.[135,136] The MEN2A mutations appeared to replace one of four cysteines with another amino acid in the extracellular domain of the protein encoded by exons 10 and 11. Families that have isolated MTC or those with the full MEN2A syndrome share the same mutations. However, there is a correlation between disease phenotype and the specific mutation (e.g., a mutation in cysteine 634 results in a high risk of pheochromocytomas). [137] Studies of individuals from multiple MEN2B patients demonstrated two specific missense mutations in the highly conserved tyrosine kinase domain of the *RET* gene[138,139,140 and 141] in more than 95% of MEN2B patients. Screening of sporadic MTC tumors have revealed a wider range of somatic mutations in the *RET* gene than those seen in the inherited cases.[142]

These findings have both scientific and clinical importance. The pattern of mutation seen in MEN2 families is not consistent with the two-hit hypothesis (reviewed by Mulligan and Ponder[142]). These specific missense mutations are not inactivating, and there is no evidence that the remaining wild-type *RET* allele is lost. Further evidence was gained by demonstrating that transfection of a *RET* allele mutated at a cysteine residue results in transformation of NIH3T3 cells. [143] Thus, current information suggests that MEN2A and MEN2B confer predisposition to cancer due to inheritance of a mutation that activates the *RET* oncogene. Conversely, in an unexpected development, inheritance of an inactivating mutation of the *RET* oncogene appears to be responsible for a small percentage of aganglionic megacolon or Hirschsprung's disease (reviewed by Martucciello[144]).

Clinically, the screening and treatment of MEN2A and MEN2B families have been significantly improved by these genetic discoveries. In comparison of DNA-based screening with calcitonin assays[145,146] there is greatly increased sensitivity and specificity in DNA testing, particularly for young children. The test need only be performed once, and it decreases the cost and morbidity associated with annual pentagastrin stimulation testing. All patients at risk for MEN2 should have molecular analysis of their *RET* gene performed. When possible, an affected member of the family can be screened first to determine the specific mutation in the family, and then at-risk individuals are screened for that particular mutation. If no affected members are available, however, the mutations are specific enough to allow direct screening of at-risk individuals with high sensitivity (particularly for MEN2B). All individuals with MTC (either sporadic or familial) should have DNA analysis performed to ascertain whether they carry a constitutional mutation in *RET* and to determine the need for screening in other family members. All children who are found to be mutation positive need prophylactic thyroidectomy by age 5 years for MEN2A and by age 1 for MEN2B. [147,148] In addition, they require lifelong surveillance for development of pheochromocytoma and parathyroid disease.

### *Atypical Teratoid and Malignant Rhabdoid Tumors and the Rhabdoid Predisposition Syndrome*

Rhabdoid tumor of the kidney is a rare, aggressive childhood cancer. [149,150] Although the infant kidney is the most common site for rhabdoid tumors, they occasionally are observed in other sites and in older children and even adults. The tumor is histologically defined by large cells of unknown origin that may resemble benign or malignant skeletal muscle cells. Some 10% to 15% of rhabdoid tumors of the kidney in infants are associated with separate primary tumors of the central nervous system.[149,150] These histologically resemble primitive neuroectodermal tumors (including medulloblastoma or pineoblastoma) or rhabdoid tumors. Because of its potential to differentiate into heterologous elements at the cellular level, this tumor type has been termed *atypical teratoid/rhabdoid tumor* (ATRT).[151]

Cytogenetic analyses of ATRTs of the central nervous system (CNS) and malignant rhabdoid tumors (MRTs) of the kidney revealed abnormalities of chromosome 22, in particular, loss of one entire copy of the chromosome or deletion or translocation involving 22q11.2. [152,153,154 and 155] In 1998, the *hSNF5/INI1* gene was isolated from chromosome band 22q11.2, and several rhabdoid tumor cell lines have been shown to harbor truncating mutations of this gene. [156] *hSNF5/INI1* encodes a protein that is part of a multi-protein complex involved in chromatin remodeling, an essential process for regulation of gene expression. Beigel and colleagues have reported *hSNF5/INI1* mutations in virtually all MRT/ATRTs examined.[157] In this study, they also reported that approximately 20% of children with apparently sporadic tumors harbored germline mutations of the gene, suggesting a potential hereditary component to the etiology of the disease. Subsequent studies by Delattre and co-workers confirmed this finding and also noted the complete loss-of-function of *hSNF5/INI1* in the tumors arising in the context of a constitutional mutation. [158] These studies suggest that this gene fulfills the features consistent with a tumor suppressor gene, namely biallelic, somatic loss-of-function mutations in sporadic tumors and constitutional alterations associated with somatic loss of the wild-type allele in tumors resulting in a dominantly inherited cancer predisposition syndrome. The proposed condition, termed *rhabdoid predisposition syndrome* may include a spectrum of tumors, including renal and extrarenal MRT, choroid plexus carcinoma,

central peripheral neuroectodermal tumor (PNET), and medulloblastoma.[158] The penetrance of this syndrome is very high at a very young age, and in most cases represents a de novo mutation in the proband with unaffected siblings or parents. Only one family to date has been reported in which the healthy mother of an infant with ATRT carries a heterozygous *hSNF5/INI1* mutation.[159] The high penetrance together with the highly fatal outcome in affected family members probably accounts for the rarity of families with multiple affected generations and the lack of recognition of this cancer frequently being due to constitutional mutation.

***Familial Leukemia: The One-Hit Model***

Acute leukemias are the most frequent malignancy of childhood. However, knowledge about genetic predisposition to leukemia is very limited compared to many less common malignancies. Some well-described autosomal dominant syndromes, including LFS and NF, demonstrate an increased risk of leukemia as one of many features as described elsewhere in this chapter. However, families that demonstrate a specific predisposition to leukemia are extremely rare (reviewed by Horwitz [3]).

Over the last 5 years, progress has been made in mapping loci responsible for these rare families, and the first specific gene has been cloned. Linkage analysis of AML families implicated at least two genetic loci responsible for these families at 9p21-22 and 16q22. [160,161] In addition, this analysis demonstrated an unusual feature of the AML families[160] in that the age of diagnosis of leukemia was significantly lower for later generations within a family. This phenomenon is termed *genetic anticipation* and is well-documented in other genetic syndromes (e.g., myotonic dystrophy) due to progressive expansion of a trinucleotide repeat sequence within the disease-causing gene. There are no cancer genetic syndromes for which triplet expansion has been demonstrated. However, other familial cancer syndromes, including neuroblastoma[162] and Hodgkin's disease,[163,164] have also been reported to demonstrate genetic anticipation. In each of these cases, the specific mutations responsible for the familial cancers have not been identified. Thus, the molecular mechanism responsible for anticipation in cancer families is not known.

Familial platelet disorder with predisposition to AML (FPD/AML) is an autosomal dominant syndrome characterized by both neonatal thrombocytopenia and a very high propensity to develop AML. Although these families are rare, the high penetrance of the platelet disorder facilitated linkage analysis that has resulted in localization of the syndrome to chromosome 21q2.[165] In 1999, the laboratory of Gilliland and colleagues reported on the isolation of the gene responsible for this syndrome.[166] Quite unexpectedly, four FPD/AML kindreds were shown to contain constitutional mutations in the *CBFA2/AML1* gene. This gene had been previously well-described to be translocated in a significant percentage of sporadic cases of AML and is considered an oncogene. [167] At first glance, this might appear to be similar to MEN2 in which activating mutations in an oncogene cause a familial syndrome. What is unique in the FPD/AML case is that the mutations reported in the four pedigrees appear to be inactivating. They include nonsense mutations, splice site mutations (resulting in a downstream frameshift), and an intragenic deletion all of which should prevent production of normal CBFA2/AML1 protein. This pattern of mutation is much more consistent with a tumor-suppressor gene. Even more intriguing is early data that the remaining copy of the *CBFA2/AML1* gene is not disrupted in the leukemic cells from these patients. The authors conclude that haploinsufficiency of *CBFA2/AML1* is sufficient to lead to tumorigenesis. Thus, AML in the FPD/AML syndrome may not require a “second hit.” This analysis has also led to a reevaluation of the impact of translocations on *CBFA2/AML1* function that are found in sporadic leukemia. [167]

***Familial Colon Cancer***

Although not generally considered a pediatric disease, children of familial colon cancer kindreds can present with gastrointestinal manifestations, including frank carcinoma in the adolescent period. In addition, there is an increased prevalence of a variety of pediatric malignancies, including hepatoblastoma and brain tumors. The familial colon cancer syndromes are divided into those associated with polyposis (i.e., familial polyposis coli) and hereditary nonpolyposis colon cancer (HNPCC).

**Familial Adenomatous Polyposis**

The classic carpeting of the colon with thousands of polyps in familial adenomatous polyposis (FAP), also known as *adenomatous polyposis coli* (APC), led to early discovery of the inherited nature of this disorder. [168,169] The major features of the syndrome include onset in the second or third decade of extensive polyposis, with a nearly 90% rate of development of malignant colorectal carcinoma in the third decade and beyond (reviewed by Haggitt and Reid [170]). In 1962, Gardner and colleagues[171] noticed extracolonic manifestations in some kindreds with polyposis, including desmoid cysts, cysts of the mandible, and osteomas. Another feature, congenital hypertrophy of the retinal pigment epithelium is a sensitive diagnostic sign for Gardner's syndrome. [172] However, with the discovery of the gene, these “different” disorders were found in some cases to be caused by identical mutations, and the distinction between Gardner's and FAP no longer appears valid.

In addition to the greatly increased risk of colorectal carcinoma, carriers of this disorder have an increased risk of upper gastrointestinal malignancies, thyroid cancer, and pediatric hepatoblastoma. The upper gastrointestinal tumors include duodenal and periampullary adenocarcinomas and can result in increased mortality in patients post-colectomy.[173,174 and 175] Other environmental or hereditary factors may influence the upper gastrointestinal malignancies; Japanese patients with FAP have a very high rate of gastric cancer that is not seen in Western patients. [174,176]

Of particular importance for pediatric oncologists, the lifetime risk of developing hepatoblastoma for children of FAP families is approximately 1 case per 250 persons, compared with 1 per 100,000 in the general population. [177,178 and 179] Although this represents a minority of children with hepatoblastoma, it is important to inquire about a family history of colon cancer and polyposis in a child diagnosed with hepatoblastoma. In addition, approximately 1% of FAP patients develop thyroid cancer, and some authors recommend beginning surveillance for this at age 15 years. [180]

The gene for FAP (called the *APC gene*), was cloned by using positional methods in 1991. [181,182 and 183] Both constitutional mutations are found in FAP kindreds as well as frequent somatic mutations in the *APC* gene in sporadic colon cancers consistent with a tumor suppressor gene that follows two-hit kinetics. [184] Carriers of *APC* mutations are detected by two methods, protein truncation assays and linkage analysis (reviewed by Nagase and Nakamura [185]). First, approximately 70% of APC mutations result in truncated proteins[186] assayed by a method specifically designed to detect shortened proteins. The particular position of the truncation mutation appears to correlate with severity of the phenotype. [187] The assay is first performed on one affected member of the family. If a shortened protein is found, family members at risk can then be studied specifically for that alteration. Alternatively, if the assay fails, linkage analysis of the family as a whole can be performed to determine the likelihood that at-risk individuals inherited the mutant chromosome 5. [174]

Unlike the situation for LFS, there are clear surveillance and prophylactic surgery guidelines for individuals found to be affected with FAP that appear to be lifesaving. Given the early age at which polyposis develops, screening by serial flexible sigmoidoscopy is recommended to begin between the ages of 8 and 10 years for mutation carriers. Prophylactic surgery that includes total colectomy with removal of the rectal mucosa is recommended after extensive polyposis develops or by late adolescence. Modern surgical techniques allow the maintenance of fecal continence in these patients. [175,188] Surgery is required whether or not polyposis develops, because many carriers of the disorder have few polyps but still develop early colorectal cancer. After prophylactic surgery, carriers need screening of their upper gastrointestinal tracts and rectums (if rectal mucosa is left in place) for development of malignancy.

Substantial research has focused on treating FAP pharmacologically. Sulindac, a nonsteroidal antiinflammatory drug, was found to reduce the number of polyps in patients with FAP.[189,190 and 191] However, the later studies demonstrated that the effect was only partial and that colorectal carcinoma can still develop. The proposed mechanism of action is through induction of apoptosis in the abnormally proliferating colonic epithelial cells. Subsequent studies with the more specific DOC-2 inhibitors have also shown efficacy. [192] Trials to treat mutation-positive children pre-symptomatically are being initiated.

**Familial Juvenile Polyposis**

Familial juvenile polyposis (JP) was originally identified as the finding of isolated or multiple hamartomatous polyps in the rectocolon of young children. These lesions often manifest with abdominal pain and rectal bleeding. [193,194] JP[195] is inherited as an autosomal dominant trait. [196,197] The controversy has centered on whether these lesions carry an increased risk of colorectal carcinoma. [196] In a review of 57 cases, ten patients had malignancies, and several other large pedigrees have shown an increased risk of malignancy,[196,197] with recent studies estimating a 50% lifetime risk of colorectal carcinoma in some affected families. [198] It is unclear if this risk is shared by children having a single, isolated hamartomatous polyp. [199] The genetic basis for JP has been found to be due to mutations in the *SMAD4* gene in a subset of families,[200,201] and genetic testing for this disorder is becoming available. Surveillance recommendations for JP include annual complete blood cell count (to detect anemia due to gastrointestinal blood loss) and semi-annual colonoscopy. Prophylactic colectomy is not recommended because the risk of colorectal cancer is lower than that seen in FAP. Other rare syndromes also associated with intestinal hamartomas include Bannayan-Riley-Ruvalcaba and Cowden syndromes, which are due to mutations in the *PTEN* tumor suppressor gene.[202,203]

### Hereditary Nonpolyposis Colon Cancer

The HNPCC syndromes were originally defined by Lynch, who in following the work of Warthin [204] observed a number of families with an increased risk of colon cancer and absence of polyposis.[205,206] Extracolonic malignancies, including uterine, ovarian, and upper gastrointestinal cancers, are seen in some kindreds. Given the reduced age of onset of cancer, these malignancies can manifest in the second decade of life. [207] In general, screening by colonoscopy is recommended to begin at approximately age 25 years, with continuing screening biannually. [208] For families with particularly early onset, Lynch recommends screening beginning 5 years before the earliest known onset of cancer in the family.[207] A recent analysis of 25 children presenting with colorectal carcinoma younger than age 18 years demonstrated a pattern of colon and uterine cancer in relatives suggestive of HNPCC. [209] Molecular studies have not yet been performed on that cohort to confirm the presence of mutations in genes implicated in HNPCC.

During the search for the genes mutated in HNPCC kindreds, an unusual DNA pattern, termed *microsatellite instability*, was identified.[210,211] The localization of the gene and the finding of microsatellite instability led to the rapid cloning of the first gene, *hMSH2*, mutated in some HNPCC families.[212,213] *hMSH2* is the human homolog of a microbial mismatch repair gene *MUTS*. Subsequent analysis has shown that mutations in three mismatch repair genes, *hMSH2*, *pML1*, and *MSH6*, are responsible for the majority of HNPCC families (reviewed by Bocker[214]). Absence of normal mismatch repair function in colonic epithelial cells leads to microsatellite instability. Although 10% to 20% of sporadic tumors in adults demonstrate microsatellite instability, [215] microsatellite instability is rarely found in pediatric malignancies with the exception of secondary malignancies[216] and brain tumors associated with Turcot syndrome.

### Turcot Syndrome: Association of Brain Tumors with Colon Cancer

Turcot and others first reported an unusual finding of multiple pediatric brain tumors in families that also had an increased risk of polyposis and colon cancer (i.e., Turcot syndrome).[217] Analysis of subsequent families revealed that some had an increased risk of gliomas and other families had an increased risk of ependymoma. The history of polyposis and colon cancer was also variable. Some families with Turcot syndrome were shown to have truncation mutations in the *APC* gene.[218] In a study of 14 families, Hamilton and co-workers[219] found that families with Turcot syndrome have mutations in *APC* (ten families) or HNPCC loci. The type of brain tumor correlated with the mutation; in the families with *APC*-related mutations, there were more medulloblastomas, with a relative risk of developing medulloblastoma 92 times (95% confidence interval = 29 to 269) that of the general population. Three families with glioblastoma multiforme had microsatellite instability in their tumor specimens, as did the original family studied by Turcot. Two of these families had detectable mutations in the mismatch repair genes *hMLH1* and *hPMS2*. Thus, the clinical phenotype of these disorders should be enlarged to include pediatric brain tumors, and careful attention should be paid to a history of colon cancer in relatives of pediatric brain tumor patients. Some authors suggest that there may be a third form of Turcot syndrome due to a recessive condition with brain tumors and polyps coexisting in affected children. Molecular evidence for a recessive form has now been found in one family in whom the children are compound heterozygotes for mutations in the *PMS2* mismatch repair gene.[220]

### *Phakomatoses*

The final group of autosomal dominant disorders that is reviewed are the phakomatoses. The word *phacomatosis* refers to multiple phacomas (Greek for tumor of the lens) and mato (Greek for spot or spotty), which refers to the patchy nature of these disorders. Although these disorders share many features of the other autosomal dominant disorders, their frequency in the pediatric population and their pleomorphic symptoms deserve additional comment.

### Neurofibromatosis Type 1

NF1 is one of the most common genetic disorders in the general population (reviewed by Gutmann and Collins [221]). Approximately 1 in 2,500 people is affected by this disorder. Table 3-5 lists the diagnostic criteria for NF1 that were formulated at a National Institutes of Health conference in 1988 and recently updated. [222] Many of the criteria, including café-au-lait spots, axillary freckling, and neurofibromas, are detectable by general physical examination. Lisch nodules, which are normally detected by slit-lamp examination, are a pathognomonic feature of NF1. They are particularly useful in diagnosing older children and adults, because the prevalence of Lisch nodules is estimated to be greater than 80% for adults older than 20 years with NF1. [223] Lisch nodules do not have any impact on vision and often are not commented on in routine ophthalmologic examination.

| |
|---|
| The diagnosis is confirmed if the patient has two or more of the following features: |
| Six or more café-au-lait spots |
| 1.5 cm or larger in postpubertal individuals |
| 0.5 cm or larger in prepubertal individuals |
| Two or more neurofibromas of any type |
| or |
| One or more plexiform neurofibromas |
| Freckling of armpits or groin |
| Optic glioma (tumor of the optic pathway) |
| Two or more Lisch nodules (benign iris hamartomas) |
| A distinctive bony lesion |
| Dysplasia of the sphenoid bone |
| Dysplasia or thinning of long bone cortex |
| First-degree relative with NF1 |

From Gutmann DH, Aylsworth A, Carey JC, et al. The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. JAMA 1997;278:51–57, with permission.

**TABLE 3-5. DIAGNOSTIC CRITERIA FOR NEUROFIBROMATOSIS TYPE 1 (NF1)**

The hallmark of NF1 is the development of benign tumors, including peripheral neurofibromas, plexiform neurofibromas, gliomas of the optic tract, other low-grade gliomas, and pheochromocytomas. The peripheral neurofibromas often do not begin to develop until adolescence and rarely cause significant cosmetic problems until adulthood.[224,225] In contrast, plexiform neurofibromas are believed to be congenital in nature and can develop within the first few years of life. [226] Plexiform neurofibromas develop most commonly in the craniofacies, paraspinous region, mediastinum, and retroperitoneum. [225,227] They are deep masses that can be covered by hyperpigmented skin. They can be invasive and can cause significant disability, depending on the structures they invade. There is little malignant potential, but an area of a plexiform neurofibroma that becomes painful or begins to grow rapidly should be investigated. There is no satisfactory treatment for these tumors; partial resection is used if they become too disabling or invade the spinal tract. Clinical studies to determine the efficacy of farnesyl transferase inhibitors have been proposed.[228]

A second benign growth that is common in early childhood is the development of gliomas, especially involving the optic tract. A large percentage (30% to 70%) of children with a new finding of optic glioma have NF1.[229,230] Conversely, approximately 15% of children with NF1 have some optic tract involvement when assayed by magnetic resonance imaging (MRI) or computed tomography scanning.[231] Approximately one-third of these children have lesions that grow large enough to interfere with vision. In general, clinically significant growth occurs in the first 6 years of life. Because of the difficulty in detecting visual changes in young children, MRI of the brain and optic pathway is often performed for a young child with NF1. However, performing scans in asymptomatic children is controversial, as has been discussed by several authors.[222,231,232] If by age 6 a child does not show any sign of optic pathway involvement, the prognosis for lack of eye involvement is excellent. [233] Treatment of optic tract gliomas that are enlarging include irradiation, chemotherapy (carboplatin based), and surgery. [230,232,234] Although treatment guidelines are controversial, several of these large series that demonstrate the optic gliomas associated with NF1 have a more favorable course over long-term follow-up.

Gliomas can also develop in other parts of the central nervous system. These range from very low-grade to high-grade malignant tumors. Indications for imaging include seizures and development of neurologic deficits. In several small studies, the presence of an optic glioma in childhood may predispose the person to the development of other gliomas,[235,236] a finding that will have to be validated by larger studies.

Because NF1 is a common disease, cases of NF1 and malignancy are likely to happen coincidentally. The clearest associations between NF1 and pediatric malignancies is the increased risk of optic gliomas and malignant peripheral nerve sheath tumors (PNST). [237,238] and [239] The increased risk also includes other

sarcomas, presumably representing malignant transformation of the benign lesions seen in most NF1 patients. A large population study from Japan of 26,084 children younger than 15 years revealed a six- to eight-fold increased incidence of cancer in NF1 patients. [237] In particular, gliomas, PNST (i.e., malignant schwannomas), rhabdomyosarcomas, and myelogenous leukemia were all significantly increased compared with the non-NF1 Japanese population. Fifty percent of the patients with malignant PNST had NF1, a percentage similar to that found in a large Dutch study. [239] The Japanese study also highlighted the fact that many physicians were unaware of the diagnosis of NF1 in these patients and that the reporting of NF1 in pediatric cancer patients increased significantly when physicians were specifically asked to identify whether the patient had NF1.

Children with NF1 have an increased risk of several myelogenous disorders, including AML, [240] myelodysplasia, and myeloproliferative syndromes. [241],[242] and [243] Children with NF1 have a higher proportion of myelogenous leukemia than lymphoid leukemia (20:9) compared with the 1:4 ratio expected for the general population. [240] Moreover, bone marrow from children with NF1 and malignant myeloid disorders shows a loss of the normal *NF1* gene in the malignant cells. [244] This finding was unexpected, because the hematopoietic system does not derive from neural crest tissue. However, studies in mouse models of NF1 demonstrate that neurofibromin regulates proliferation of hematopoietic precursors. [245] Thus, *NF1* appears to be a tumor suppressor gene with regard to malignant myeloid disease.

The gene *NF1*, found at 17q11.2, was cloned in 1990 based on positional methods. [246],[247] and [248] The *NF1* gene is quite large and encodes a protein, neurofibronin, which is homologous to the GTPase-activating protein called GAP. This relationship suggests that the NF1 protein normally inhibits the activity of the Ras protein (an oncogene). NF1 follows the two-hit hypothesis in that tumors associated with NF1, such as pheochromocytomas, [238] show a loss of the remaining normal copy of the *NF1* gene. Evidence suggests that the benign neurofibromas also demonstrate mutation or loss of the normal copy. [249] Discovery of the loss of the second *NF1* gene in these lesions completed an almost 30-year cycle from when Nicholls [75] first presented his hypothesis of two genetic events occurring in the same gene, one inherited and one somatic, in the development of NF1.

Although the gene for NF1 has been identified for several years, translation of this knowledge into practical molecular testing has been difficult. Because of the high de novo mutation rate in NF1, most individuals have different mutations, called *private mutations*, scattered throughout a large gene. Early DNA diagnostic testing relied on linkage analysis for markers near 17q11 in those families with enough affected individuals. [250] A priori, this testing could not be used on the 50% of affected individuals who represent de novo mutations or on individuals from small families with few living affected family members. Molecular testing based on the same *in vitro* transcription-translation process as described for FAP is available to identify mutations that result in a truncated neurofibronin protein. [186] Between 60% and 70% of individuals with NF1 may have detectable mutations by this method. More comprehensive sequence-based methods are not yet clinically available. Although most individuals have their NF1 condition diagnosed based on the clinical features described in Table 3-5, molecular testing can be useful in some clinical situations. The first is affected adults requesting prenatal diagnosis (although this currently requires linkage analysis). The second is parents of affected children who appear to have new mutations. In that case, molecular testing is done on the child, and if a mutation is identified, the parents are tested to confirm that they do not carry the mutation. However, negative skin and eye examinations would have already made the likelihood fairly low. A third clinical scenario relevant to pediatricians is a child with a negative family history and multiple café-au-lait spots. This is a common reason for referral to genetics or NF clinics. Children are often examined on an annual basis, but the diagnosis remains uncertain until the child develops another sign of NF1. For these children, a positive molecular diagnostic study would provide strong confirmation of the diagnosis and highlight the surveillance required. One series of 42 young children originally evaluated for café-au-lait spots found that 24 were eventually given the clinical diagnosis of NF1. [251]

### Neurofibromatosis Type 2

NF2 represents a distinct and much rarer disorder than NF1. Because most of the manifestations of NF2 occur in adulthood, they are not discussed in detail here. NF2 is characterized by café-au-lait spots, bilateral vestibular schwannomas, central neurofibromas, and meningiomas (reviewed by Gutmann [222]). The disease has a high degree of morbidity and is difficult to treat because of the multiple tumors that develop. The gene that is mutated in NF2 is found on chromosome 22 and was cloned by positional methods in 1993 (reviewed by Kinzler and Vogelstein [252]). The *NF2* gene encodes a protein, called *merlin* or *schwannomin*, that is homologous to the band 4.1 protein and appears to play a role in cytoskeletal architecture. This represents an unusual function of a tumor suppressor gene and highlights the many aspects of cellular physiology that are involved in tumor development. Children from families with NF2 can be screened by MRI to look for evidence of acoustic nerve tumors. Consideration for teaching of sign language while hearing is intact may aid in communication if deafness develops.

### Tuberous Sclerosis

Tuberous sclerosis (TS) is another phakomatosis disorder that is characterized by pleomorphic features, including benign and neoplastic growths. The classic triad of seizures, mental retardation, and facial angiofibromas (previously called *acne sebaceum*) occur in fewer than 50% of patients with TS. [253] The diagnosis of TS is made clinically and is based on specific criteria. [253] There is a wide range of phenotypes between and within families, [254] with some adults with TS having very high degrees of intelligence. Two-thirds of cases are due to de novo mutations and thus do not have a family history of the disease. Part of the explanation for the heterogeneity may result from the fact that TS is a genetically heterogeneous disorder, with families showing linkage to at least two genes. [255] A locus on chromosome 9q34, named *TSC1*, is implicated in some TS families. The gene mutated on chromosome 9, *TSC1*, was identified in 1997. The function of the protein encoded by *TSC1,* hamartin, is not known. A second gene on chromosome 16p13.3, called *TSC2*, was cloned previously [256] and encodes a protein, tuberin, which has Rag1-Gap activity. [257] Hamartin and tuberin can physically interact. Thus, the protein products of genes mutated in both NF1 and TS participate in the regulation of Ras or Ras-related GTPase-activating protein activity and provides a molecular explanation for the similarities between the two syndromes. A comprehensive analysis of mutations in *TSC1* and *TSC2* in 150 TS patients was recently published. [258] In total, 120 mutations were found, 22 in *TSC1* and 98 in *TSC2.* The majority of *TSC1* mutations were truncating, whereas for *TSC2* there were both missense mutations in the conserved domains and truncating mutations. Clinically, the degree of mental disability was greater for patients with *TSC2* mutations (67% versus 31%).

TS is characterized by the growth of normally benign tumors in several different organs. Loss of heterozygosity for TSC1 and TSC2 has been shown in these tumors, suggesting both have tumor suppressor gene function. [259],[260] Cardiac rhabdomyomas normally develop *in utero* and are often detected during prenatal ultrasound. They typically regress postnatally. [261] The morbidity and mortality associated with these tumors reflect the potential for flow abnormalities in the heart if these tumors grow large enough. In one study, 50% of children with cardiac rhabdomyomas developed clinical criteria for TS during childhood. [262]

Later in childhood and early adulthood, individuals with TS are at risk for the development of retinal hamartomas and giant cell astrocytomas. [253] During adulthood, there is often the slow growth of renal angiomyolipomas. In the British study of childhood cancer, [1] TS was found to be significantly overrepresented in this population. The increase in cancers appears to be due to an increased risk of brain tumors and rhabdomyosarcomas. There are no specific screening guidelines for detection of tumors in TS other than renal ultrasounds periodically in adulthood to screen for renal involvement.

### von Hippel-Lindau Disease

Unlike TS, the hallmark of von Hippel-Lindau (VHL) disease is development of multiple benign and highly malignant tumors. In contrast to the other phakomatoses, this disorder is not accompanied by specific dermatologic or developmental abnormalities. Diagnosis occurs during adolescence or early adulthood, when tumors become clinically apparent. VHL is characterized by four common tumor types: multiple cerebellar hemangioblastomas, retinal angiomas, renal cell carcinoma, and pheochromocytomas. [263],[264] The affected members of these families also have increased rates of pancreatic carcinoma and epididymal cysts. The two leading causes of mortality are the cerebellar lesions and renal cell carcinoma. [264]

The cerebellar and retinal lesions typically develop during the second and third decade of life, although they can occur in the first decade. Presentation is related to the location and mass effect of the tumor. [264],[265] MRI of both the brain and spinal cord can reveal the presence of isolated or multiple lesions with signal intensities characteristic of a hemangioblastoma. Any person with a central nervous system hemangioblastoma should undergo complete evaluation for other VHL-related tumors. Multiple cerebellar hemangioblastomas or a first-degree relative with VHL and an isolated lesion is sufficient for the diagnosis of VHL.

Treatment options include surgery and irradiation. Retinal angiomas can often be asymptomatic and diagnosed on yearly eye examinations. If sufficient in size, they can manifest with new vision defects. Treatment of the retinal lesions can yield excellent long-term results. [264]

Renal cysts accompanied by renal cell carcinoma are one of the hallmarks of the VHL syndrome. The tumors often develop in the third or fourth decade, but the risk of renal cell carcinoma is lifelong. It is necessary to balance curative intent, tumor removal, maintenance of renal function, potential for transplantation, and the

knowledge that the patient is likely to develop other tumors when creating a treatment plan. [266]

Pheochromocytoma as part of VHL can be singular or multiple and may be benign or malignant. One study of a series of individuals with sporadic pheochromocytoma found 23% with evidence for VHL or MEN2A syndrome.[267] Although this high rate of genetic predisposition was not found in other studies, [268] all patients with pheochromocytoma should have a careful family history taken and screening for signs of involvement of other organs consistent with either VHL or MEN2. In VHL, the pheochromocytomas most commonly present in the second decade but have been reported in young children.

Given the predilection in VHL to develop a specific group of tumors, several comprehensive screening protocols have been developed. Table 3-6 outlines the protocol recommended by the large VHL research program at the National Institutes of Health.[263] The important features are annual surveillance examinations for renal masses, pheochromocytoma, and retinal angiomas, with biannual examination for cerebellar lesions. Screening for pheochromocytoma is improved by use of plasma metanephrines[269] as opposed to urine catecholamines. Outcome studies are needed to document decreased morbidity, mortality, and expense for individuals with VHL who are properly screened with the current technology.

| Test | Schedule |
|---|---|
| Urinary catecholamines or plasma metanephrines | From age 2; every 1–2 yr |
| Ophthalmoscopy | From infancy; yearly |
| Enhanced MRI of the brain and spine | From age 11; every 2 yr |
| Abdominal ultrasound | From age 11; yearly until age 20 or during pregnancy |
| Abdominal CT or MRI | From age 20; yearly or every other year |

CT, computed tomography; MRI, magnetic resonance imaging.
Adapted from Choyke PL, Glenn GM, Walther MM, et al. von Hippel-Lindau disease: genetic, clinical and imaging features. Radiology 1995;194:629–642; and Eisenhofer G, Lenders JW, Linehan WM, et al. Plasma normetanephrine and metanephrine for detecting pheochromocytoma in von Hippel-Lindau disease and multiple endocrine neoplasia type 2. N Engl J Med 1999;340:1872–1875.

**TABLE 3-6. RECOMMENDED SURVEILLANCE REGIMENS FOR VON HIPPEL-LINDAU DISEASE**

The *VHL* gene was cloned in 1994 using positional methods. [270] The original localization of *VHL* to chromosome 3 was based on the finding of frequent loss of heterozygosity for markers on 3q of patients with sporadic renal cell carcinoma. [271] VHL families with pheochromocytoma (referred to as *type 2*), tend to have clustering of missense mutations in specific codons. [272] DNA diagnostic assays have been optimized such that more than 95% of patients with VHL have a detectable mutation in the *VHL* gene.[273] This allows early identification of relatives who have not inherited the mutation and do not require a surveillance protocol and those who have inherited the mutation and need full screening for development of malignancy.

Initial analysis of the *VHL* gene revealed little homology to other known proteins. Subsequently, significant progress has been made in defining the functions of the VHL protein[274] including repression of hypoxia-induced genes (e.g., *VEGF*) at normal oxygen tension. The loss of VHL function results in overexpression of genes required for angiogenesis under normal oxygen conditions. VHL protein also has ubiquitin protein ligase activity when bound to the elongin and cullin complex. [275] Currently, experimental therapeutics are being developed to inhibit these activities.

### Nevus Basal Cell Carcinoma Syndrome or Gorlin-Goltz Syndrome

Gorlin and Goltz described a number of individuals who had multiple nevoid basal cell epitheliomas, odontogenic jaw cysts, and bifid ribs, with the syndrome being inherited in an autosomal dominant fashion.[276] Sometimes referred to as the *fifth phakomatoses*,[277] Gorlin-Goltz syndrome is currently named *nevus basal cell carcinoma syndrome* (NBCCS) due to the predominant finding of multiple basal cell carcinomas. Herzberg described these findings in concert with medulloblastoma.[277] The full syndrome includes the above features and characteristic palmar and plantar pits; mild facial dysmorphisms, including frontal and biparietal bossing; calcification of the falx cerebri; and short fourth metacarpal bones (reviewed by Gorlin [278,279]). Careful clinical examination and x-rays of ribs, skull, and spine is often sufficient to make the diagnosis. In young children with medulloblastoma, examination of the parents may aid in identifying NBCCS in the family. Basal cell carcinomas develop around the time of puberty and can eventually number in the hundreds. There are differences in the number of basal cell nevus syndromes in different racial groups, with significantly fewer found in individuals of African-American descent. [280] It is estimated that 29% of individuals with a basal cell carcinoma younger than age 18 years has NBCCS syndrome.

Meduloblastoma is a significant feature of NBCCS.[281] Analysis of 105 patients with NBCCS evaluated at the National Institutes of Health found four children with the diagnosis of medulloblastoma diagnosed at a mean age of 2.3 years.[280] Conversely, it is estimated that approximately 10% of patients with medulloblastoma diagnosed at age 2 years or younger have NBCCS.[282] Because of the high frequency of medulloblastoma, children with NBCCS are recommended to have biannual neurologic examinations and annual MRI examinations up to age 7 for early detection of medulloblastoma. [280]

In children receiving radiation therapy, the skin within the field can become severely affected with hundreds of nevi with a latency of approximately 5 years. [280] There have also been reports of secondary meningiomas and ependymomas in the radiation field of children with NBCCS. [280,283] Thus, use of radiation therapy for treatment of tumors in NBCCS syndrome should be limited.

The gene for NBCCS syndrome was first localized by linkage analysis and subsequently cloned. [284,285] The gene responsible for NBCCS, *PTCH,* is a homolog of the *Drosophila melanogaster* segment polarity gene Patched. Mutations in *PTCH* are found in the majority of NBCCS families and in a large percentage of sporadic basal cell carcinomas, making it one of the most frequently mutated genes in human cancers.[286] Mouse models heterozygous for *ptc* mutations also develop medulloblastoma.[287] In contrast, analysis of sporadic medulloblastomas have identified *PTCH* mutations in only approximately 10% of cases.[288] Much research is now focused on whether somatic mutations in other human genes in the *PTCH* pathway are prevalent in sporadic medulloblastoma.[289] One recent study from the Mayo clinic did not find mutations in other *PTCH* pathway genes (including *SHH, SMO*, and others) in a series of 24 sporadic medulloblastomas.[290]

### Autosomal Recessive Disorders

Autosomal recessive genetic disorders that predispose to cancer have distinct characteristics when compared with the autosomal dominant disorders. Autosomal recessive disorders are much rarer in the general population. Specific ethnic or geographic groups may have an increased risk of autosomal recessive disorders because of a founder effect or increased prevalence of consanguinity. Given the requirement for two mutant alleles, these disorders normally occur in sibships and are not evident in multiple generations. Within a sibship, there is only a one in four chance that a sibling will have the disorder. For this reason, single affected individuals from a small family may appear to be a sporadic case.

Generally, the range of expressivity in an autosomal recessive disease is more limited and the symptoms often more severe than in autosomal dominant disorders. However, exceptions include the wide range of presentations of Fanconi's pancytopenia. [291] Most of these disorders manifest in childhood, presumably because of the severe nature of the genetic defect. Many of the autosomal recessive cancer syndromes are caused by mutations in genes that encode DNA repair enzymes or DNA damage checkpoint genes, and they are often referred to as *chromosome breakage syndromes*. These deficiencies result in increased sensitivity to spontaneous and exogenous DNA damage and increased risk of specific cancer types. Significant progress has been made in the last 10 years identifying the genes mutated in these disorders.

#### *Xeroderma Pigmentosum, Cockayne Syndrome, and Trichothiodystrophy*

Xeroderma pigmentosum (XP) represents the classic DNA repair defect syndrome. The clinical features of this disorder have been extensively reviewed by Kraemer and colleagues.[292,293] Patients present with cutaneous sensitivity, as revealed by photosensitivity, telangiectasias, and freckling in the first few years of life. Ocular abnormalities are common and are found in ultraviolet light-exposed areas of the cornea, lids, and conjunctivae, including corneal clouding and ocular malignancies.

There is a several thousand–fold increased risk of basal and squamous cell skin carcinomas, which begin developing at approximately 8 years of age ( Fig. 3-3), compared with at approximately the age of 50 for the general U.S. population. There is also a significant increase in melanoma (approximately 5% lifetime risk) in the XP group. The pattern of these skin cancers shows an increased prevalence in sun-exposed areas compared with non-XP skin cancer patients, particularly on the neck, face, and tip of the tongue. A significant but smaller increase in the risk of internal malignancies has also been observed for XP patients. [292]

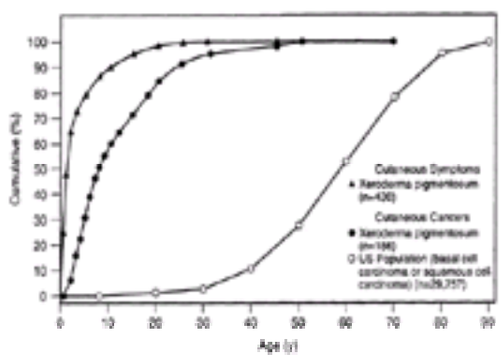


**FIGURE 3-3.** Age of onset of xeroderma pigmentosum symptoms. The ages at onset of cutaneous symptoms (generally sun sensitivity or pigmentation) was reported for 430 patients. The age at diagnosis of the first skin cancer was reported for 186 patients and is compared with distribution for 29,757 patients with basal cell carcinoma and squamous cell carcinoma in the U.S. general population. (From Kraemer KH, Myung L, Scotto J. Xeroderma pigmentosum. Arch Dermol 1987;123:241, with permission.)

Some XP patients have neurologic abnormalities. One group, first reported by DeSanctis and Cacchione, has XP-like dermatologic features and progressive neurologic degeneration beginning at approximately the age of 2 years and accompanied by immature sexual development. [294] These patients tend to cluster in complementation group A. Overall, Kraemer[293] found that 18% of reported XP patients had neurologic abnormalities, some of which resemble the DeSanctis-Cacchione syndrome, and others that have a later onset of neurologic difficulties and that cluster in complementation group D.

Two other disorders can manifest with findings of XP. Trichothiodystrophy is a rare disorder that shares an increased risk of skin cancer and repair defects and the findings of brittle hair and ichthyosis. The XP/trichothiodystrophy patients fall in the XP complementation group D. [295] Cockayne syndrome shares some of the ultraviolet hypersensitivity of XP but is also characterized by neurologic deficits, including developmental delay and decreased skin cancer risk. There are at least three complementation groups for Cockayne syndrome.[296]

At a cellular level, the ultraviolet sensitivity [297] in XP patients was found to result from defects in excision repair.[298] This form of repair is essential for repair of the thymine dimers and other structures that result from ultraviolet damage.

XP is a group of disorders caused by mutations in at least seven different genes. [295] The determination that multiple genes caused the same clinical disorder was based on complementation assays in which fibroblasts from different patients are fused together and the heterokaryon cell is then assayed for complementation of the repair defect (reviewed in Bootsma and Hoeijmakers[299]).

The specific biochemical defects in cells from patients with either XP or Cockayne syndrome has led to successful isolation of almost all of the genes responsible for this defect (reviewed by Cleaver[300]). Most commonly, complementation cloning was performed based on adding either normal genomic DNA, complementary DNAs, or protein extracts to a mutant cell line and screening for the rare clone that has regained normal excision repair. Using a variety of these different but related techniques, the genes for complementation groups XPA-XPG have been found. Analysis of these gene products demonstrate that many of them are related to previously identified DNA repair genes in other eukaryotes, including yeast, mouse and Chinese hamster cells. [301]

Research on Cockayne syndrome has led to a greater understanding of the repair process and its interaction with transcription. Fibroblasts from Cockayne patients appear to have normal levels of general DNA repair.[302] In a normal cell, DNA damage in actively transcribed genes is preferentially repaired before DNA from inactive parts of the genome, a process termed *transcription coupled repair*. Cells from Cockayne patients are deficient in transcription coupled repair due to a mutation in the *ERCC2* DNA helicase, which is also a component of active transcription complex TFIIH.[303] The overlap between transcription and repair functions may explain the more extended phenotype of Cockayne patients, including neurologic dysfunction (reviewed by Cleaver and Hultner [304]). Also the fact that overall repair rates are normal in Cockayne cells may explain the lack of cancer predisposition.

A third group of patients who clinically demonstrate ultraviolet sensitivity but have normal nucleotide excision repair *in vitro* are termed *XP*$^{v}$. This disorder is due to mutation of a specialized DNA polymerase, polymerase eta, which places two adenine residues opposite a thymine dimer photoproduct, thus restoring the normal base sequence.[305,306]

***Helicase Disorders: Bloom, Werner, and Rothmund-Thomson Syndrome***

Three autosomal recessive disorders, although distinct, share some clinical features, including a predisposition to malignancy ( Table 3-7). Children with Bloom syndrome are very small at birth and remain small,[307] have a photosensitive rash, immunodeficiency, and a very high predisposition to develop a wide variety of malignancies, including leukemia/lymphomas and solid tumors.[308] This disorder is more common in children of Ashkenazi descent. Cells from these patients exhibit increased recombination manifested as increased sister chromatid exchange. Werner syndrome is characterized by premature aging (including early-onset atherosclerosis, diabetes, and cataracts beginning in the second decade) with increased incidence of soft tissue sarcomas. [309] The premature aging is manifested at a cellular level as early senescence in fibroblasts from these patients. The third disorder in this group, Rothmund-Thomson syndrome (RTS), is characterized by a very distinct rash termed *poikiloderma* that begins in infancy; skeletal dysplasias, including radial ray abnormalities; and cataracts. Children with RTS have a distinct predisposition to the development of osteosarcoma and, less frequently, skin cancers. [310] No specific cellular defect has been consistently reported in this condition.

| Disease | Clinical features | Cancer predisposition | Gene/ chromosome location |
|---|---|---|---|
| Bloom | Small stature, photosensitive rash, immunodeficiency | Multiple tumor types, including leukemia/lymphoma and solid tumors | *BLM* 15q26.1 |
| Werner | Premature aging, cataracts, diabetes, atherosclerosis | Soft tissue sarcoma and skin cancers | *WRN* 8p11 |
| Rothmund-Thomson | Poikiloderma rash, radial ray defects, cataracts | Osteosarcoma and skin cancers | *RECQL4*? 8q24.3 |

[a]Although *BLM* and *WRN* are the genes mutated in the majority of Bloom and Werner syndrome patients, respectively, to date, only a few patients with Rothmund-Thomson syndrome have been documented to have mutations in *RECQL4*.

**TABLE 3-7. FEATURES OF THE CHROMOSOME INSTABILITY SYNDROMES DUE TO MUTATIONS IN GENES ENCODING RECQ HELICASES**

All three disorders have been shown to be the result of mutations in RecQ helicase genes: the *BLM* gene in Bloom syndrome,[311] *WRN* gene in Werner syndrome,[312] and *RECQL4* in a subset of patients with RTS.[313] The RecQ helicases were first identified in *Escherichia coli.* The most extensive studies have been of

*Saccharomyces cerevisiae* strains mutant for the *SGS1* RECQ helicase gene. These strains show genomic instability, including a hyperrecombination phenotype that may explain the chromosomal instability seen in the human disorders.[314]

***Ataxia-Telangiectasia***

Ataxia-telangiectasia (AT) is a disorder that has fascinated physicians and researchers since its initial description (reviewed by Gatti and colleagues [315]). Children with AT develop ataxia during early years of childhood, with truncal ataxia appearing before appendicular and eventually requiring a wheelchair for mobility. [316] Choreoathetosis and ocular motor apraxia are also common neurologic findings. Intelligence does not appear to be affected. The oculocutaneous telangiectasias normally begin with the conjunctivae and develop between the ages of 3 and 5 years. Useful biochemical markers for diagnosis include elevated alpha-fetoprotein and carcinoembryonic antigen in children with AT.[317]

There is a very high rate of malignancy, particularly the development of leukemias [318,319] and lymphomas, in AT children. Although less emphasized, these AT homozygotes are at increased risk of many different types of solid tumors, including primary central nervous system tumors (reviewed by Hecht and Hecht [319]). Individuals with AT have immunodeficiency characterized by diminished immunoglobulin G2 and immunoglobulin A levels and increased risk of sinopulmonary infections.[315] The major causes of mortality of those with this syndrome are sinopulmonary infection (especially after significant neurologic degeneration) and malignancy (reviewed by Gatti[315]).

In addition to the risk of cancer in the AT homozygous children, heterozygotes carrying one AT mutation appear to have an increased risk of cancer. Swift and colleagues carried out several studies of AT heterozygotes and demonstrated increased cancer risk, particularly for breast cancer, in AT heterozygotes compared with the general population.[320,321] Although the degree of increased breast cancer risk continues to be controversial (despite a large number of studies) [322] mothers of children with AT should be advised of at least a moderate increased risk of breast cancer.

The many cellular defects in AT cells have been intensively studied and are not reviewed here in detail. Fibroblasts and lymphocytes from AT patients have increased sensitivity to DNA-damaging agents, particularly ionizing radiation. [323,324] and [325] In general, this defect results from the loss of checkpoint control, the cell cycle arrest in G1 and G2 that normally accompanies DNA damage.[326]

The gene mutated in AT (called *ATM*) was sought for more than 10 years by complementation studies and positional cloning. The positional studies, which involved several international consortia, centered on using genetic linkage and physical mapping to identify the *ATM* gene. AT was localized to chromosome 11q22-q23 in 1988 by Gatti and colleagues.[327] In 1995, the group led by Yossi Shiloh identified a complementary DNA from this region that was mutated in multiple AT patients. [328] Subsequent studies have revealed that the mutations are spread throughout the gene and primarily result in a truncated or disrupted ATM protein.

The *ATM* gene encodes a protein with homology to the *MEC1/ESR1* gene of budding yeast and the *Rad3* gene of fission yeast. Mutations in these genes result in a defect in checkpoint control in the S and G2 phases of the cell cycle, with remarkable similarity to the defect seen in AT cells (reviewed by Elledge [329]). Subsequent analysis has found that after DNA damage, the ATM protein signals through the p53 and BRCA1 tumor suppressor gene products. [330] The finding of ATM in the same molecular pathway as other genes clearly implicated in breast cancer susceptibility further substantiates the epidemiologic data with regard to breast cancer predisposition in heterozygotes. Although specific screening or surveillance for cancers is not currently recommended for children with AT, the diagnosis of AT has a major impact on treatment decisions for a child who develops a malignancy. Due to the defective DNA damage checkpoint mechanisms, children with AT have increased sensitivity to chemotherapy and radiation treatments. Specific treatment regimens have been developed for these children. [331] Specialized clinical centers that are familiar with recommended regimens for these unique children are available through the support of the A-T Children's Project ( http://www.atcp.org/).

## ISSUES IN GENETIC TESTING FOR THE PEDIATRIC ONCOLOGY PATIENT

Several studies have suggested that 4% to 10% of childhood cancers result from inherited genetic mutations, making it essential for pediatricians and pediatric oncologists to recognize clinical criteria suggestive of familial cancer syndromes. [332,333] As we have discussed in this chapter, specific features in the clinical history are essential for the accurate definition of a familial cancer syndrome. Most of these features rely on an accurate and detailed family history, including all cancers and their age of diagnosis. This is because many cancer predisposition syndromes lack an associated recognized phenotype to identify at-risk individuals. For example, although HH and other features of BWS raise the clinician's alertness to embryonal cancer risk, no known physical features are associated with LFS. As oncologists have become more effective at identifying cancer families, and as novel genes functionally linked to cancer phenotypes are identified and clinical testing made available, it is important to continue to recognize that children are part of a network of family members who may be indirectly affected by testing.

In the practice of pediatrics, DNA-based tests for a large number of non-cancer conditions, including cystic fibrosis, muscular dystrophy, and hemophilia, have been developed and are currently in use. It has been recommended that as children grow and acquire cognitive and moral skills, they should be permitted to participate in decisions concerning testing.[334] For genetic testing for conditions associated with childhood-onset cancers, it is generally accepted that cancer predisposition testing is most helpful for highly penetrant diseases in which individuals at risk for cancer can be identified and followed closely for the development of highly specific tumors (e.g., VHL, FAP, and the multiple endocrine neoplasias). [335] For each of these, clear guidelines for clinical surveillance or prophylactic medical interventions in childhood have been established for mutation carriers as discussed in this chapter.

However, for a variety of other cancer-predisposition disorders, the clinical management of carriers is less well-defined. Such diseases include LFS. Although predisposition testing may identify asymptomatic carriers, and allow institution of preventive or surveillance programs where available, such testing is associated with the following caveats that must be taken into consideration: (a) the genetic heterogeneity of cancer predisposition, (b) the technical difficulty inherent to gene testing and to test interpretation, and (c) the psychosocial impact of testing. Both variable degrees of penetrance and expressivity for many conditions, including LFS, suggest that other genetic events play an important role in defining the particular cancer phenotype of individual members of families. This variability makes predictions of clinical disease and specific susceptible target organs difficult and complicates the design of adequate screening programs.

The technical aspects involved in predisposition gene testing and interpretation are complex. Many tests are only available through research settings where results are made less immediately available, and confirmation of results is less well controlled than in clinically certified laboratories. Databases are now available to facilitate identification of laboratories performing specific genetic tests (e.g., http://www.genetests.org/). Furthermore, such testing, particularly of novel genes, tends to be expensive, and extra effort by the physician often needs to made to obtain insurance coverage of testing. Given the complexity, genetic testing should only be undertaken by a physician or genetic counselor fully capable of interpreting these results. As demonstrated for FAP a significant percentage of physicians ordering a genetic test incorrectly interpreted a negative result in an affected proband. [336]

Genetic testing for any disease, which should now include cancer, has been demonstrated to have profound psychological and emotional impact on patients, and may be further complicated by relationships with parents and other family members.[337] Issues of the “vulnerable child syndrome” in affected carriers and “survivor guilt” in unaffected, non-carrier siblings raise complex psychosocial concerns that may be beyond the general purview of the pediatric oncologist. Furthermore, lessons from studies in adults have demonstrated that although patients learning of their increased risk of disease do well overall they may experience feelings of shock, depression, grief, altered self-esteem, or even guilt. Limited studies in children, parents, and families have yet to clarify the impact of predictive testing for cancer in children.

Based on many of the preceding arguments, a number of recommendations established in 1992 for LFS[338] are still applicable to genetic testing in family cancer syndromes that include children. The quality of information provision on cancer genetics is directly related to the knowledge of professionals and their ability to communicate this to a patient and family regardless of their specialty.[339] This requirement exists in the face of a relative lack of in-depth education in genetics in medical schools and post-graduate education, that then places pediatricians and pediatric oncologists in a difficult position of integrating rapidly evolving technologies with patient care and unfamiliar and complex genetic testing issues.[340] This unfamiliarity extends to more recent issues with respect to physicians' duty to warn “third parties” (i.e., members of extended families who may be at risk of avoidable harm from a genetically transmissible condition), and its legal ramifications. [341] Therefore, for pediatric oncologists without additional training, it is reasonable and preferable to identify appropriate patients and families for referral to a geneticist or genetic counselor with training in cancer genetics. Recently, the multi-disciplinary approach taken by several groups [342] involving pediatric oncologists, clinical geneticists, genetic counselors, psychologists, and ethicists in establishing cancer genetics clinics and programs whose primary focus is to serve children with cancer and their families provides an intriguing and novel mechanism to optimize care of these families and advance the understanding of the role of genetics in the etiology of

childhood cancer.

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# 4

# MOLECULAR BASIS OF CHILDHOOD CANCER

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ILAN R. KIRSCH



## INTRODUCTION

The genetic information found in a cancer cell is not the same as that in its nonmalignant counterpart. This realization, and the generation of the data that support it, constitute one of the successes of the molecular approach to cancer biology. This knowledge represents the resolution of a search begun more than a century ago to define and identify something unique to a tumor cell that could distinguish it from the surrounding normal cells in which it arose. The search has been arduous and frustrating, but now we know that cancer cells are different. They carry within their DNA point mutations, viral insertions, or gene amplifications, deletions, or gene rearrangements, each of which can alter the context and process of normal cellular growth and development. Depending on the genetic locus involved and on the mechanism of its disruption, some of these changes may make small or incremental contributions to malignant transformation. Other changes may be cataclysmic in their unraveling of an ordered and regulated growth process. The identification and characterization of the involved genes and of the mechanisms by which they can be altered provide basic insights into the process of carcinogenesis and offer the hope of specific therapies if the alteration or its effect can be stymied or reversed. At the most fundamental level, cancer is a malevolent example of genomic instability, an inherent property of the evolutionary process and normal human development.

The results of genomic instability are not abstract or intangible. They can be readily characterized by current methods of DNA analysis. In every case, they result in a change in a chromosome. Some changes can only be appreciated by analysis of the fine structure of the chromosome, such as by comparing a nucleotide sequence one base at a time with the corresponding sequence from a nonmalignant cell. The point mutations revealed can terminate protein translation, alter protein function, or change the regulatory target sequences that control gene expression. Other changes cause chromosomal alterations that generate morphologically distinct structures that can be appreciated by refined cytogenetic analysis. These aberrations create new genetic contexts within the genome, leading to the formation of novel proteins or to the dysregulation of genes displaced by the aberrant event.

Although the genome in each person is inherently unstable, that instability can be increased or decreased through inheritance and exposure to destabilizing factors in the environment. Certain inherited syndromes are characterized by a marked increase in the tendency for chromosomes to break and rejoin or by a marked predisposition to develop point mutations. Invariably, these syndromes are associated with marked increases in cancer incidence. Even in the absence of a frank cancer-predisposing syndrome, humans have inherited a panoply of genes that govern the ability to cause, recognize, or repair mismatched or altered nucleotides and chromosome breaks. Similarly, each person carries genes whose products can detoxify mutagens or increase their mutagenic potential. Interactions with the environment—locations of homes, types of occupations, medicines taken, and food eaten—affect the stability of the genome and the likelihood of acquiring a gene mutation that can move a cell along a pathway toward malignant transformation.

With this perspective in mind, we can begin to consider the specific nature of the genetic alterations that are the basis of cancer. To begin this discussion, we review events that result in a fundamental alteration of the normal linear organization of genetic material, gross chromosomal aberrations, a particularly dramatic and instructive form of genomic instability. These aberrations result in a change in the “neighborhood” in which particular genes are located as opposed to changes restricted to the gene's primary structure. For most of the past 30 years, delineation of these chromosomal aberrations has focused on changes that could be observed in metaphase chromosome preparations analyzed by light microscopy. Advances in cytogenetic and molecular biologic technologies have vastly improved our ability to detect and resolve the precise consequences of chromosomal aberrations.

## GENERAL NATURE OF CANCER-ASSOCIATED CHROMOSOMAL ABERRATIONS

Figure 4-1 shows the general classes of chromosomal alterations that have been associated with malignant transformation. The occurrence of these defects or changes is not restricted to malignant cells. Gaps, breaks, monosomies, trisomies, deletions, and translocations occur in nontransformed cells and can be observed as incidental findings during karyotypic analysis of normal individuals. Their frequency is a function of the age of the individual, the individual's exposure to DNA-interactive agents, the cell type being studied, and whether the cell is being studied directly or after *in vitro* cell culture.

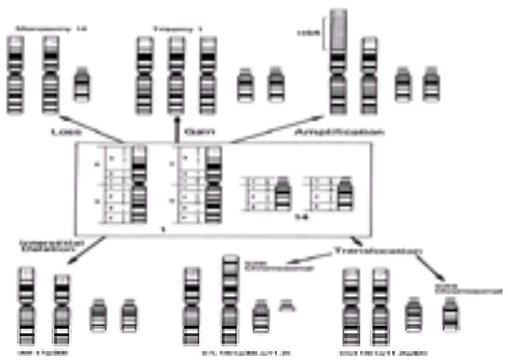



**FIGURE 4-1.** The spectrum of morphologically apparent gross chromosomal aberrations, with chromosomes 1 and 14 as examples.

The frequency of any incidental abnormality in a routine karyotypic analysis of a normal population of cells hovers at approximately 1%. [1,2,3,4] and [5] These karyotypically abnormal cells that are observed incidentally do not appear to be clonally proliferative (by definition, they are unique in a given analysis) and therefore are assumed not to confer any selective advantage on the cell in which they occur. Whether they are completely random or have some cell-type specificity is not entirely clear. Certainly, a fraction of the recurring chromosomal translocations and inversions in normal individuals are cell-type specific. A background level of chromosomal instability exists. Rarely, a chromosomal aberration occurs that provides a growth or selective advantage to the affected cell and dysregulates the growth of the cell and starts it down a pathway of malignant transformation. Individual aberrations do not appear to exert their dysregulating effect uniformly, but they seem to have variable effects depending on the cell type in which they occur. A gene product must already be part of a growth-affecting pathway of a particular cell or be able to insinuate itself into that pathway for its dysregulation to have an impact. This lesson is one derived from cancers associated with constitutional and acquired karyotypic defects.

## CONSTITUTIONAL CHROMOSOMAL ABERRATIONS

When chromosomal deletions, translocations, amplifications, point mutations, or nondisjunction events occur in a gamete, the abnormality exists in the germline, and the entire organism that develops after conception bears the alteration in each and every cell. A variation on this theme can occur when the alteration or nondisjunction event occurs in a somatic cell early in its lineage development, leading to mosaicism for the entire organism or for particular cell types. The frequency of generation of chromosomal abnormalities is high enough that everyone harbors chromosomally abnormal cells, and everyone is mosaic. Usually, mosaics are appreciated only when the aberration occurs and expands early in development, and only those abnormalities that occur during the initial formation of cell lineages or in the germline are considered to represent constitutional abnormalities.

Constitutional deletions or monosomies can predispose an individual to the development of cancer. That the cancers are cell-type specific underscores the fact that one is often dealing with a gene function that only makes a contribution to growth or development within a particular milieu or physiologic context. The classic examples of this phenomenon taken from the pediatric oncology literature are the chromosomal abnormalities associated with the hereditary forms of retinoblastoma and Wilms' tumor (nephroblastoma). The consequences of the disruption of the *RB* gene in retinoblastoma and of *WT1* and other genes in Wilms' tumor are discussed in a later section of this chapter, but the fundamental observations are presented here.

Hereditary and sporadic occurrences of retinoblastoma[6] have been distinguished on the basis of clinical and epidemiologic presentations. The hereditary form (i.e., familial or de novo germline mutation) is estimated to comprise approximately 40% of affected individuals. In this form, the age of onset is earlier and the frequency of bilateral tumors is increased. There is often a positive family history for this cancer. These observations led Knudson [7] to propose a “two-hit” mechanism of carcinogenesis in which the first genetic defect, already present in the germline, must be complemented by an additional spontaneous mutation before a tumor can arise. In the sporadic form, cellular transformation occurs only when two spontaneous mutations take place in the same cell. [7] Support for this concept came from karyotypic analysis of patients with a particular syndrome associated with retinoblastoma. These individuals carried a constitutional deletion of part of the long arm of one allele of chromosome 13.[8,9] This finding not only fit conceptually with the Knudson model but also pointed directly to the chromosomal region in which the crucial gene was likely to be found.

The involvement and often deletion of one of the two alleles from this region of chromosome 13 in patients with retinoblastoma was proven by molecular analysis of restriction endonuclease fragment length polymorphisms. The somatic, unaffected tissue of a particular patient with retinoblastoma was studied with a series of DNA probes from the 13q14 region that show a polymorphic heterozygous pattern (with each allele on the two chromosomes 13 contributing its own distinctive pattern) on Southern blot analysis (Fig. 4-2). The tumor tissue was likewise analyzed, and the contribution of one of the two alleles was found to be missing. There had been a “reduction to homozygosity,” consistent with an acquired monosomy in the tumor tissue.[10] These kinds of analyses continued to focus attention on a particular chromosomal region and led to the successful cloning of the first tumor suppressor gene, *RB*.[11]

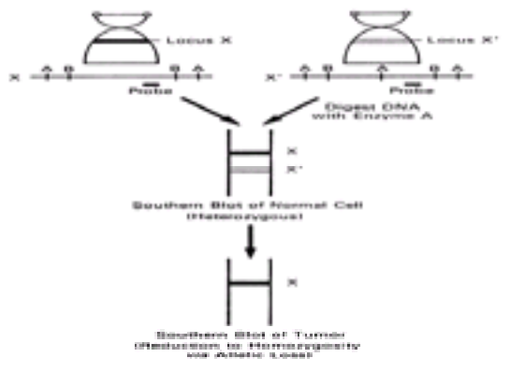



**FIGURE 4-2.** Polymorphic variation and reduction to homozygosity. A locus “X” is polymorphic for a DNA sequence, which is the target site for cutting of the restriction endonuclease “A.” The individual in this example carries one copy of each of two polymorphic variants. After digestion of the individual's genomic DNA, size fractionation through a gel matrix, denaturation, and transfer and fixation to a solid matrix (i.e., Southern blotting procedure), the “blot” is hybridized to a labeled probe that is homologous to a part of the locus. In normal cells, DNA fragments corresponding to both alleles (and therefore polymorphic variants) are seen. In the DNA derived from the tumor of this individual, only one of the two DNA fragments is observed. The other allelic variant has been lost. This loss can occur by simple deletion of the locus or chromosome carrying the other allele, by loss of one and reduplication of the other locus, by point mutation of the polymorphic site, or by gene conversion. In any case, there has been a reduction to homozygosity of the DNA for this particular locus ( *locus X*).

The fact that familial retinoblastoma exists at all is, to some extent, the result of a medical success. A highly penetrant familial childhood cancer syndrome could not have existed during previous generations of human development. Such affected individuals would have died from their malignancies and never reached reproductive age. Only when individuals harboring *RB* mutations in the germline had their retinal tumors treated effectively so that they reached reproductive age and passed the mutation on to their offspring did the entity of familial retinoblastoma become manifest. Essentially, all patients who present with bilateral or trilateral (i.e., affecting the pineal body) disease carry a germline mutation of the *RB* gene, but this mutation may have been inherited from a parent or generated de novo in the patient. An exception to this scenario has been appreciated with the recognition of families that present with the phenotype of partially penetrant retinoblastoma. In these families, familial predisposition is clearly evident by pedigree analysis, but tumors may not always be present or, if present, may be predominantly unifocal in obligate carriers. In many of these families, the *RB* alteration has been demonstrated to be caused by mutation that leads to an unstable temperature-sensitive protein. These selected amino acid changes cause fluctuation in the ability of *RB* to interact with its relevant binding partners. [12]

Another example of a constitutional chromosomal aberration providing insight into a disease entity followed from an analysis of the presentation and family history of

patients with Wilms' tumor. A rare syndrome of Wilms' tumor consisting of aniridia, genitourinary defects, and mental retardation (WAGR) was found to be correlated with a constitutional deletion of chromosome band 11p13,[13] and a Wilms' tumor susceptibility gene, *WT1*, has been cloned from this region.[14,15] It encodes a DNA-binding transcription factor whose expression in fetal kidney and embryonic structures suggests its involvement in genitourinary development.[16,17] Patients with another rare syndrome associated with a chromosomal abnormality distinct from the WAGR syndrome also show an increased susceptibility to the development of Wilms' tumor. These individuals with the Beckwith-Wiedemann syndrome (i.e., macroglossia, somatic gigantism, visceromegaly, hypoglycemia, and abdominal wall defects), often have constitutional duplications of the 11p15 region, and their Wilms' tumors manifest a reduction to homozygosity of marker DNA segments in this region, although not in 11p13.[18] There is evidence that genomic imprinting occurs within this region (see the section Genomic Imprinting), and thus differential loss of a duplication of the active allele may contribute to tumorigenesis.[17] Candidate loci for this event include IGFII, H19, and p57 $^{KIP2}$. Other chromosomal regions implicated in Wilms' tumor include 16q, 1p, 7p, and the p53 gene on 17p. Thus, there appears to be more than one Wilms' tumor predisposition gene.

**Genomic Imprinting**

Part of the complexity of the etiology of neoplasms such as Wilms' tumor may be derived from a de facto inactivation of one of the two alleles of certain genes in the absence of a structural deletion or alteration of the locus. The inactivation is imposed because of the origin of the gamete from which it was derived. For example, there can be a cellular “memory” about whether a particular chromosome was derived from the egg or sperm, and the two chromosomes, or at least certain genes that reside on them, may be activated or suppressed differentially. It represents a constitutional difference in the expressability of the two genes derived from homologous chromosomes in the individual. There may be nothing wrong with the structure of a gene itself, but it may not be expressed in a particular individual because it resides on the maternally or paternally derived chromosome. That the gene itself is completely functional can be demonstrated by following its movement into the opposite type of gamete. For example, a gene not expressed on the paternally derived chromosome but transcribed normally when passed through the female germline has the opportunity to be transferred to the next generation through the female ovum, and because the gene is then maternally derived, it is functional in the offspring ( Fig. 4-3).

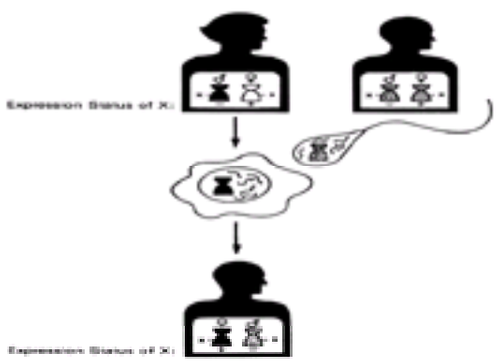


**FIGURE 4-3.** Genomic imprinting. The activity of a gene can be a function of which parent donates (i.e., imprints) it. In this example, a woman carries a particular gene “X,” one copy of which comes from her father (*male symbol*) and one from her mother (*female symbol*). The gene from her father is inactive only because it was inherited from her father; nothing is inherently wrong with it. The gene donated by her mother is active. This woman produces an ovum within whose haploid complement of chromosomes is the gene X inherited from her father. The egg is fertilized by a sperm that similarly carries a haploid chromosomal constitution. Together, they generate a diploid human organism. The specific allele that had been inactive in this offspring's mother (because it had come from her father) is active in the offspring because it was derived from his mother.

Genomic imprinting is being invoked to explain a variety of phenomena that appear to violate a simple mendelian model of inheritance. One can appreciate how this effect can complicate cancer genetics, because a particular predisposition to develop a specific kind of cancer (e.g., Wilms' tumor) may not always show linkage with a particular aberrant gene (e.g., one on chromosome 11p13 or 11p15) but rather may be related to whether the otherwise normal gene was derived from the patient's mother or father. This kind of situation does seem to be relevant to Wilms' tumor,[19,20,21] and[22] osteosarcoma,[23] and embryonal rhabdomyosarcoma. In one study, normal and tumor tissue from six patients with embryonal rhabdomyosarcoma and normal tissue from their parents were analyzed with DNA marker probes from chromosome 11. A reduction to homozygosity was seen in each tumor; invariably, it was the paternally derived chromosome 11 that had been retained in the tumor tissue.[24] These data led the investigators of this study to propose a modification of the Knudson model of tumorigenesis. They suggested that aberrations of cancer-related genes because of their imprint can lead to a constitutional lack of expression from one allele, thereby contributing to an inherited predisposition to certain types of cancer. In Beckwith-Wiedemann syndrome, also an example of the impact of genomic imprinting, there is a correlation between the presence of two paternal copies of the 11p15.5 region and the development of this disorder.[25] Although not completely understood, the mechanism by which a gene is imprinted seems to reside in complex coordination of *cis*-regulatory elements and differential methylation.[25,26,27,28,29] and[30]

**Trisomy**

The monosomic form of chromosomal aneuploidy was discussed previously in the context of a two-hit model of carcinogenesis, which involves a genetic or epigenetic knockout of a functional growth-affecting gene. Conceptually, the impact of that kind of monosomy may be easier to envision than the impact of trisomy resulting in the addition of one extra copy of a particular gene or genes. Trisomies in the germline result in dramatic phenotypic variance from normal growth and development and are for the most part incompatible with life, an intolerance that is much more acute in mammals than in other genera.[31,32] Only trisomies of chromosomes 13, 18, and 21 occur with any frequency in the germline of viable human beings, and each is associated with a defined syndrome.[33]

It is possible that an unbalanced number of chromosomes by itself causes a problem for the cell during normal cell division, but this seems unlikely given the vigorous growth of aneuploid cell lines *in vitro*. It would seem more likely that the presence of an extra chromosome poses a basic developmental problem due to a 50% increase (for autosomes) in the dosage of a particular gene or genes. In many instances, there can be a wide tolerance by the organism for twofold variation in the dosage of particular genes, as in the many recessive syndromes in which obligate heterozygotes are phenotypically normal. The impact of trisomies underscores the fine tuning and dose dependency of certain growth and developmental pathways in higher organisms.

The classic constitutional aneuploidy that demonstrates predisposition to certain kinds of cancer is trisomy 21, also called *Down syndrome*.[34,35] and[36] An immediate question is whether the increased risk of leukemia in Down syndrome is directly related to a gene or genes on chromosome 21 or is an indirect effect of some other aspect of the Down phenotype. It would appear that the former may be the case. In trisomy 21 mosaics, it is the trisomic cell that is at risk for leukemic transformation, and acquired trisomy 21 is a relatively frequent chromosomal abnormality found in acute lymphocytic or nonlymphocytic leukemias.[37,38,39] and[40] Acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) can occur in patients with Down syndrome[41]; acute megakaryoblastic leukemia may be the most common leukemia seen in these patients.[42,43]

A different but probably related hematopoietic disorder, transient leukemoid reaction (TLR), is also associated with Down syndrome, or trisomy 21 mosaicism, although it is not restricted to this population.[44,45,46] and[47] TLR classically manifests in newborns or early infancy as a myeloproliferative disorder that can include hepatosplenomegaly, leukocytosis, and circulating myeloblasts; the morphologic picture is consistent with congenital leukemia except that spontaneous remission occurs. This condition is not unequivocally benign, however; 20% to 30% of Down syndrome patients with TLR develop overt megakaryoblastic leukemia at 1 to 3 years of age.[48]

With the development of extensive high-resolution genomic maps that unite cytogenetic landmarks with the rough draft of the human sequence,[49,50] there is no dearth of potential growth-affecting genes at almost any chromosomal location, including those on chromosome 21. A critical region and candidate Down syndrome genes have been identified on chromosome 21.[51,52] Genes relevant to chromatin structure, lymphocyte adhesion, interferon action, DNA transcription, and signal transduction reside on chromosome 21, but none has been implicated in the constitutional phenotype or leukemogenic risk of patients with Down syndrome.[53] Through the use of restriction fragment length polymorphism analysis, it is similarly possible to determine the origin of the extra chromosome 21 in Down syndrome. A DNA polymorphism present on a maternal chromosome 21 is present in two copies in the affected offspring. In trisomy 21, this analysis has demonstrated that the

extra chromosome 21 is of maternal origin in approximately 95% of cases.[54] Scattered reports based on small numbers of patients seem to suggest that, among individuals with TLR or leukemia, there is an increased paternal contribution of the extra chromosome 21. If this observation is confirmed in additional studies, it will suggest that more complex genetic and epigenetic factors may influence the risk of leukemia or TLR among patients with trisomy 21. [55]

### Sex Chromosome Abnormalities and Neoplasia

The clearest example of a sex chromosome abnormality associated with neoplasia is Klinefelter's syndrome. The classic sex chromosome constitution of these individuals is 47,XXY. The presence of the Y chromosome confers phenotypic maleness on these individuals, but they may also have atrophy and dysgenesis of the seminiferous tubules and palpable breast tissue. [56] An association exists between Klinefelter's syndrome and breast carcinoma,[57,58] with a 20-fold increased risk of developing this tumor compared with that in the normal male population. Individuals with Klinefelter's syndrome also have an increased incidence of germ cell tumors.[59]

Gonadoblastoma and dysgerminoma can be associated with Turner's syndrome, although not in cases with the classic 45,XO karyotype. Those with karyotypic variants in which a part or all of the Y chromosome is present are the population at particular risk for these tumors. [56] The increased risk of tumorigenesis in those with Klinefelter's syndrome or in a subset of individuals with Turner's syndrome may result in part from inappropriate hormonal stimulation, from germ cell developmental defects, or perhaps from a combination of the two. The answer will come with better molecular definition of the character and interaction of the sex-determining genes on the X and Y chromosomes, gene dosage effects, and the targets of action of these genes.

## SOMATICALLY ACQUIRED CHROMOSOMAL ABERRATIONS

For the past 30 years, cytogeneticists have been describing an increasing number of specific chromosomal abnormalities, each associated with particular cell types or histologically distinct malignancies. These abnormalities differ from those discussed in the previous section in that they are confined to the malignant clone and not found in the normal tissues from the same individuals. There is a continuum from a constitutional chromosomal abnormality present in every cell in the patient's body, to constitutional mosaicism in which an abnormality may be present in a large proportion of the patient's cells or tissues, and to those abnormalities found only in a particular tissue or cell type. The main distinguishing features are the stages of development in which they occur and whether they are capable of being transmitted vertically from generation to generation. The kinds of constitutional abnormalities discussed in the previous section occur in every cell (more or less) but are only phenotypically significant in a subset of cell types. For example, patients with constitutional abnormalities of chromosome band 13q14 show a predisposition to the development of retinoblastoma and osteosarcoma, although not rhabdomyosarcoma. Cell-type–specific or cancer-specific abnormalities are often more directly correlated with a phenotypic effect. When found, they seem to be of significance to that cell lineage; they are seldom observed in cells in which their expression has no importance. This raises the issue of whether there is something about the state of a particular cell type in terms of chromatin accessibility, nuclear matrix proteins, or transcriptional regulation that predisposes the cell to the development and selection of a particular chromosomal aberration.

The association of a particular chromosomal abnormality with a specific type of malignancy began in 1960 with the identification of the Philadelphia chromosome in the malignant cells of patients with chronic myelogenous leukemia.[60] This chapter focuses on the chromosomal aberrations that have been associated with malignancies that particularly affect the pediatric population, although there is overlap with malignancies that also affect adults, and the lessons derived from cytogenetic analyses of one population are relevant to the other. However, the distinctiveness of many of the chromosomal aberrations reiterates the fact that childhood cancers often differ from cancers in older individuals because of the progenitor cells involved and the mechanisms of malignant transformation.

## PROTO-ONCOGENE CONVERSION

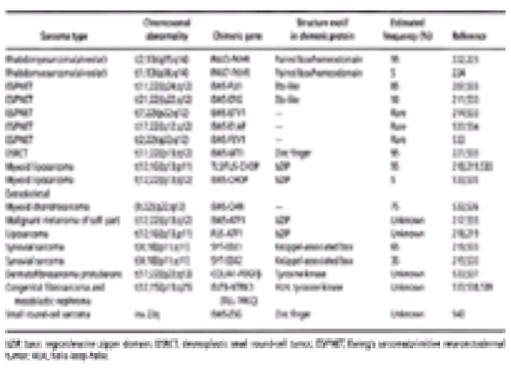
The cancers of childhood are ultimately the products of somatically acquired genetic abnormalities that modify protein function. The classes of proteins affected by these changes include growth factors and their receptors, kinase inhibitors, signal transducers, and transcription factors, which may act dominantly as cellular oncoproteins or recessively through loss of function, as in the case of tumor suppressors. Studies of the structure and properties of dominantly acting oncoproteins have contributed importantly to our understanding of the molecular events that initiate and sustain the neoplastic state. The general paradigm emerging from this work is that certain types of molecular lesions activate or repress key regulatory genes whose abnormal expression may be tumorigenic in specific hematopoietic and mesenchymal progenitors. Most candidate oncoproteins are poorly characterized with regard to their mode of action; however, their remarkable similarity to proteins involved in the earliest stages of invertebrate development suggests important roles in the integration of pathways regulating the growth, differentiation, and survival of normal and neoplastic cells.[61]

### Activation of Transcriptional Control Genes by Chromosomal Rearrangement

Disruption of transcriptional control genes is a common mechanism by which chromosomal rearrangements contribute to the genesis of acute leukemias and sarcomas (Table 4-1 and Table 4-2). Such genes are preferred targets because their transcription factor products bind to regulatory elements in DNA, such as promoters and enhancers, where they stimulate or sometimes inhibit gene transcription and the expression of messenger RNA. More than 80% of these proteins can be classified on the basis of recurring structural motifs within their DNA- and protein-binding domains, designated as basic region/helix-loop-helix (bHLH), basic region/leucine zipper (bZIP), zinc finger, and homeodomain (helix-turn-helix; Fig. 4-4); other motifs with similar functional significance are termed *A-T hook, Ets-like*, *runt homology*, and *cysteine-rich (LIM)*.[61,62] and [63] The modular organization of transcription factors provides an ideal framework for their multiple functions, particularly binding to DNA in heterodimeric complexes.[64] It also explains why disruption and rearrangement of transcriptional control genes by chromosomal translocations can produce functional hybrid proteins rather than inert peptides.



### TABLE 4-1. ALTERED TRANSCRIPTIONAL CONTROL GENES THAT CONTRIBUTE TO THE ACUTE LEUKEMIAS



| Sarcoma type | Chromosomal abnormality | Chimeric gene | Structure motif in chimeric protein | Estimated frequency (%) | Reference |
|---|---|---|---|---|---|
| Rhabdomyosarcoma(alveolar) | t(2;13)(q35;q14) | PAX3-FKHR | Paired/fkhrhomeodomain | 55 | 132,133 |
| Rhabdomyosarcoma(alveolar) | t(1;13)(q36;q14) | PAX7-FKHR | Paired/fkhrhomeodomain | 5 | 134 |
| ES/PNET | t(11;22)(q24;q12) | EWS-FLI1 | Rna-like | 85 | 109,133 |
| ES/PNET | t(21;22)(q22;q12) | EWS-ERG | Rna-like | 10 | 111,133 |
| ES/PNET | t(7;22)(p22;q12) | EWS-ETV1 | -- | Rare | 174,133 |
| ES/PNET | t(17;22)(q12;q12) | EWS-E1-a8 | -- | Rare | 135,154 |
| ES/PNET | t(2;22)(q33;q12) | EWS-FEV1 | -- | Rare | 133 |
| DSRCT | t(11;22)(p13;q12) | EWS-WT1 | Zinc finger | 95 | 137,155 |
| Myxoid liposarcoma | t(12;16)(q13;p11) | TLS/FUS-CHOP | bZIP | 95 | 141,143,133 |
| Myxoid liposarcoma | t(12;22)(q13;q12) | EWS-CHOP | bZIP | 5 | 133,155 |
| Synovial sarcoma | t(X;18)(p11;q11) | SYT-SSX1 | Kruppel-associated box | 65 | 139,133 |
| Synovial sarcoma | t(X;18)(p11;q11) | SYT-SSX2 | Kruppel-associated box | 35 | 139,133 |
| Congenital fibrosarcoma and mesoblastic nephroma | t(12;15)(p13;q25) | ETV6-NTRK3 (EIL-TRK3) | HLH tyrosine kinase | Unknown | 135,138,139 |
| Small round-cell sarcoma | inv (16) | EWS-FEV | Zinc finger | Unknown | 140 |

bZIP, basic region/leucine zipper domain; DSRCT, desmoplastic small round-cell tumor; ES/PNET, Ewing's sarcoma/primitive neuroectodermal tumor; HLH, helix-loop-helix.

### TABLE 4-2. CHIMERIC TRANSCRIPTIONAL CONTROL GENES IN THE CHILDHOOD SARCOMAS

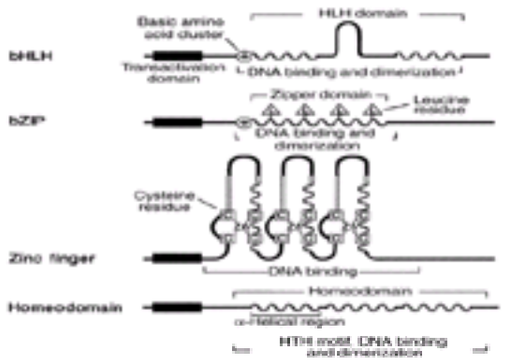



**FIGURE 4-4.** Structural motifs characterizing the major oncogenic transcription factors in childhood cancer. Each defining structure—basic region/helix-loop-helix (bHLH), basic region/leucine zipper (bZIP), zinc finger, and homeodomain—functions in DNA binding, protein dimerization, or both. +, positive charge; C, cysteine; HTH, helix-turn-helix; L, leucine; Zn, zinc. (Adapted from Papavassiliou AG. Molecular medicine: transcription factors. N Engl J Med 1995;332:45.)

In an effort to develop a unifying hypothesis that would account for the tumorigenicity of altered transcription factors, Rabbitts [62] emphasized similarities between these oncogenic proteins and the products of so-called master genes, which specify lineage-specific patterns of gene expression during embryologic development. Most intriguing are the similarities between the conserved regions of mammalian transcription factors and those of developmental proteins regulating *Drosophila* embryogenesis (Table 4-3).[65,66 and 67] Current observations suggest that aberrant transcription factors may act positively to up-regulate critical target genes or negatively to interfere with normal regulatory cascades that coordinate the expression of proteins required to complete cell differentiation. [61,68]

| Human protein | DNA-binding domain | *Drosophila* protein |
|---|---|---|
| HLF | Basic region/leucine zipper | Giant |
| PLZF | Zinc finger | Krüppel |
| AML1 | Runt homology | Runt |
| PAX3 | Paired box | Paired |
| FKHR | Forkhead | Sloppy-paired |
| HOXA9 | Homeobox | Antennapedia |
| PBX1 | Homeobox | Extradenticle |
| MLL | A-T hook | Trithorax |

Adapted from Look AT. Oncogenic role of "master" transcription factors in human leukemias and sarcomas: a developmental model. In: Vande Woude G, ed. Advances in cancer research. San Diego: Academic Press, 1995:25–55.

**TABLE 4-3. EXAMPLES OF STRUCTURAL MOTIFS SHARED BY ONCOGENIC TRANSCRIPTION FACTORS AND PROTEINS REGULATING MORPHOGENESIS IN *DROSOPHILA* EMBRYOS**

Chromosomal translocations modify transcription factors in one of two ways. Most often, the DNA-binding, dimerization, and *trans*-effector regions of discrete proto-oncogenes are "stitched together" to produce a chimeric transcription factor with altered function. A second consequence is the dysregulated expression of intact transcription factor coding sequences caused by their relocation to sites near the promoter/enhancer elements of T-cell receptor (TCR) or immunoglobulin genes. The transcription factors involved in leukemia and sarcoma pathogenesis have unique transforming properties that are specific for the different types of progenitors within these two developmental pathways (illustrated for the leukemias in Fig. 4-5).

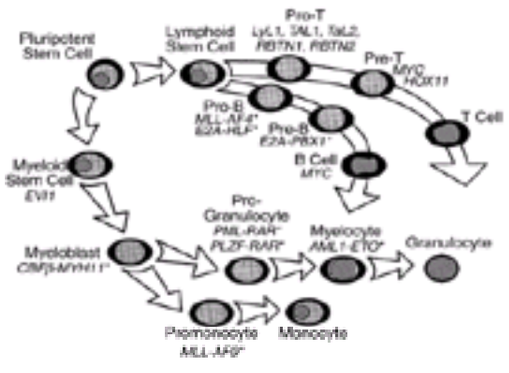



**FIGURE 4-5.** Hematopoietic cells transformed by dysregulated transcription factor genes. A recurring theme in research on oncogenic transcription factors is their specificity for early stages of the myeloid and B- or T-lymphoid cell lineages. The pattern of activation shown here supports the concept that such genes contribute to malignancy by specifically disrupting normal programs of cell differentiation. Hyphenated genes ( *asterisk*) are created by translocation-mediated fusion events. (From Look AT. Oncogenic roles of "master" transcription factors in human leukemias and sarcomas: a developmental model. In: Vande Woude G., ed. Advances in cancer research. San Diego: Academic Press, 1995:25–55, with permission.)

### *TEL-AML1 Fusion Gene in Early B-Lineage Acute Lymphoblastic Leukemia*

Although the most common cytogenetic abnormality found in children with ALL is the t(12;21)(p13;q22), this translocation is missed by conventional cytogenetic analysis because the rearranged chromosomal fragments closely resemble the morphology of the involved chromosomes. When analyzed by molecular approaches (fluorescent *in situ* hybridization, Southern blotting, or reverse transcriptase-polymerase chain reaction), the t(12;21) is found in approximately one-fourth of pediatric B-cell ALL cases,[69,70] and approximately 3% to 4% of adult ALL cases.[70] This rearrangement results in the fusion of the oligomerization domain of *TEL (ETV6)* on chromosome 12 to the entire coding region of *AML1 (CBFA2)* on chromosome 21 (Table 4-1).[71] Both of these genes are also involved in different translocations in both lymphoid and myeloid malignancies. TEL contains a dimerization motif conserved in the ETS family of proteins and has been identified in fusion with many different partners, such as *TEL-PDGFRb* in chronic myelomonocytic leukemia; *TEL-MN1*, *TEL-ABL*, and *TEL-EVI1* in AML; and *TEL-JAK2* in ALL.[71] *AML1 (CBF2A)* on chromosome 21 encodes a protein that closely resembles the *Drosophila* runt protein[72] and also is involved in the pathogenesis of AML through its fusion with the *ETO* gene in AML cases with the t(8;21).[73]

The loss of the normal *TEL* allele is frequently observed in t(12;21) $^{+}$ ALL, suggesting that TEL-AML1 (ETV6-CBFA2) requires a second genetic lesion and that TEL loss of function may contribute to leukemic transformation.[74] The vast majority of ALL cases with *TEL-AML1* (*ETV6-CBFA2)* rearrangement belongs to a favorable age group (age 1 to 9 years), with 70% to 80% diagnosed in patients aged 3 to 6 years. [75] Immunologically, the presence of this translocation is associated with a CD10 $^{+}$ early B-cell progenitor phenotype, an increased frequency of CD13 and CD33 myeloid-associated antigen expression, [76] KOR-SA3544 negativity, and a pseudodiploid karyotype.[77]

Cases of ALL with the t(12;21) have a good prognosis independent of clinical risk factors, such as age and white blood cell count at presentation. [69,78,79] These patients have a superior clinical outcome, with relapse-free survival rates approaching 90% in studies testing a variety of drug regimens, [69,78,79,80] and [81] especially those based on L-asparaginase and antimetabolites.[78] Some doubt about the prognostic value of the t(12;21) has been raised by analysis of this abnormality in relapsed ALL cases, however, because an unexpected high frequency of the *TEL-AML1* translocation (20% to 24%) has been observed in several series of relapsed lymphoblastic leukemias.[82,83] and [84] Other groups, however, report only a 10% incidence of this fusion in their relapsed cases. [75,85,86] This discrepancy is likely explained by differences in the efficacy of the induction and maintenance regimens used by the study groups. Notably, the best results with *TEL-AML1*$^+$ ALL were obtained in clinical trials of the Dana-Farber Cancer Institute Consortium, which included intensive L-asparaginase treatment.[86] In two other studies, *TEL-AML1* fusion conferred a favorable outcome only in patients who received intensive chemotherapy. [87,88] Additional prospective studies are needed to determine the true prognostic significance of the *TEL-AML1* fusion gene.

### *E2A-PBX1 Fusion Gene in Pre–B-Cell Acute Lymphoblastic Leukemia*

The *E2A* gene, located on chromosome 19, band p13.3, encodes a bHLH transcription factor. Its fusion with the *PBX1* homeobox gene on chromosome 1 as a result of the t(1;19)(q23;p13), which occurs in approximately 5% of childhood ALL cases ( Table 4-1), yields several species of chimeric proteins that differ in their extreme carboxyl ends due to differential messenger RNA splicing. [89,90,91] and [92] E2A-PBX1 hybrids retain the amino-terminal *trans*-activation domain of E2A but not its bHLH region, which is replaced by the homeobox DNA-binding and protein-protein interaction domain of PBX1. Thus, the gene targets of E2A-PBX1 are probably those specified by the homeodomain of PBX1; the homeodomain is an approximately 60–amino-acid motif first identified in *Drosophila* homeotic selector (Hom) proteins that regulate segment identity during embryogenesis.[93]

Evidence suggests that acquisition of the E2A *trans*-activation domain by PBX1 converts the latter into a positive regulator of gene transcription in lymphoid cells, which normally do not express this protein. [94,95] and [96] However, the E2A-PBX1 hybrid may not regulate transcription of downstream target genes by itself; it may function through a heterocomplex with specific homeobox proteins, analogous to the regulatory complex formed by the extradenticle protein with a subset of Hom proteins in *Drosophila*.[93,94,97,98] and [99]

Reports of E2A-PBX1 involvement in human disease have been restricted to ALLs with a pre–B-cell phenotype. However, Kamps and Baltimore [100] induced AML in lethally irradiated mice repopulated with bone marrow stem cells that had been infected with recombinant retroviruses containing *E2A-PBX1* fusion genes. Further oncogenic versatility is suggested by the induction of thymic lymphomas in transgenic mice harboring *E2A-PBX1* genes in the germline.[101] In these animals, lymphopenia involving T and B cells preceded malignant transformation, suggesting that E2A-PBX1 proteins can induce apoptosis in murine lymphocyte precursors. The failure to induce pre–B-cell leukemias in these experimental systems may reflect a heightened sensitivity of the murine lymphoid compartment to E2A-PBX1–induced programmed cell death.

Occasionally, the t(1;19) occurs in less mature (pro-B) rather than the usual pre-B (cytoplasmic µ heavy chain–positive) lymphoblasts, raising questions about its molecular repercussions in leukemias with an atypical phenotype. Privitera and co-workers [102] analyzed 17 cases of t(1;19)-positive ALL using reverse transcriptase polymerase chain reaction to amplify junctional sequences from leukemic cell RNA. Characteristic E2A-PBX1 chimeric transcripts were identified in 10 of the 11 pre-B cases but in none of the six with a pro-B phenotype, all of which lacked evidence of E2A-PBX1 fusion at the genomic level or by analysis of protein expression. These findings suggest that the genes affected by a t(1;19) in B-lymphoid progenitors depend on the cells' developmental status.

### *E2A-HLF Fusion Gene in Pro–B-Cell Acute Lymphoblastic Leukemia*

*E2A* gene elements are also involved in fusion events instigated by the t(17;19)(q22;p13) translocation, which juxtaposes the amino-terminal sequences of E2A (including the *trans*-activation domain) with the DNA-binding and protein dimerization region of the protein encoded by the hepatic leukemia factor gene ( *HLF*; Fig. 4-6). Assigned to a specific subfamily of the bZIP superfamily of transcription factors, [103,104] the HLF product is normally expressed predominantly in the liver, brain, and kidney but not in lymphoid cells. It bears significant homology to both DBP, [105] an albumin gene promoter D box binding protein, and thyrotroph embryonic factor, which *trans*-activates thyroid-stimulating hormone b expression during anterior pituitary development. [106] Leukemias that express E2A-HLF share a number of clinical and biologic features: disease onset in early adolescence, pro-B immunophenotype, hypercalcemia, and disseminated intravascular coagulation. Although relatively rare, the E2A-HLF–associated leukemias have a poor prognosis, even when treated with intensive, multi-agent chemotherapy.

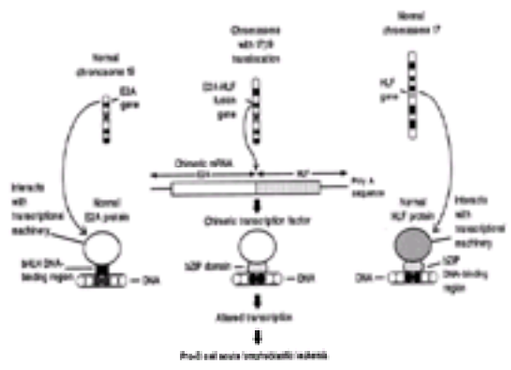


**FIGURE 4-6.** Schematic diagram of the E2A-HLF chimeric transcription factor formed in pro-B lymphoblasts by action of the 17;19 chromosomal translocation. As shown, the fusion event combines the *trans*-activation domain of the E2A protein with the basic region/leucine zipper (bZIP) DNA-binding and dimerization domain of HLF, a member of the bZIP family that normally regulates gene expression in hepatic, brain, and renal (but not lymphoid) cells. Evidence suggests that the hybrid protein binds to DNA sequences normally recognized by HLF, or perhaps a close homologue, to disrupt regulation of vital developmental programs in pro-B cells. bHLH, basic region/helix-loop-helix; mRNA, messenger RNA. (Adapted from Look AT. Oncogenic roles of “master” transcription factors in human leukemias and sarcomas: a developmental model. In: Vande Woude G, ed. Advances in cancer research. San Diego: Academic Press, 1995:25–55.)

Studies have identified a 10–base-pair DNA consensus sequence, characterized by a dyad-symmetric motif, that mediates the specific binding of both HLF and E2A-HLF proteins.[107] and [108] The chimera appears to bind DNA preferentially as a homodimer in leukemic cells, [107] suggesting that its regulatory activities may not depend on cross-dimerization with other bZIP proteins. The tumorigenicity of E2A-HLF has been demonstrated in NIH-3T3 cells and requires the *trans*-activation domain of E2A and the leucine zipper dimerization domain of HLF. [109]

Taken together, these findings suggest that the E2A-HLF fusion protein binds as a homodimer to its consensus sequence in the promoter or enhancer regions of downstream target genes, where it aberrantly regulates transcriptional programs controlling the growth, differentiation, or survival of early B-cell progenitors. Research to identify the targets of E2A-HLF binding has yielded provocative clues. In the BAF3 line of interleukin 3–dependent murine pro-B cells, overexpression of the fusion protein prolonged cell survival after withdrawal of interleukin 3, suggesting a primary effect on genes responsible for the prevention of cell lineage-specific apoptosis.[110]

### *MLL Fusion Genes in Leukemias with 11q23 Rearrangements*

An extraordinarily diverse group of chromosomal translocations and deletions affect the q23 band of chromosome 11, accounting for as many as 10% of all acute leukemias in children and adults[111] and as many as 85% of secondary leukemias in patients treated with topisomerase II inhibitors. [112,113] Of the more than 20 different chromosomal loci that have been identified as fusion partners in 11q23 translocations, [114] the most common resides on chromosome 4q21 (Fig. 4-7). This site has been implicated in approximately 4% of cases of childhood ALL overall and one-third of such cases in infants. [115]

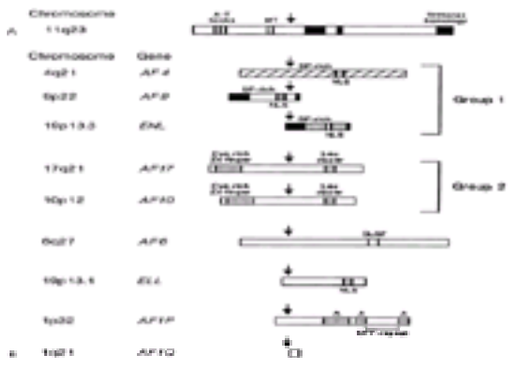


**FIGURE 4-7.** The *MLL* gene and some of its fusion partners. Clearly one of the most versatile proto-oncogenes in the acute leukemias, *MLL* can combine with at least 20 different partner genes to give rise to transformed myeloid or lymphoid progenitors. **A:** The major structural features of *MLL*, including the A-T hook and mammalian DNA methyltransferase (MT) regions, which uniformly are retained in fusion products, as indicated by the characterized breakpoint ( *arrow*). **B:** Nine of the genes known to participate in fusion events with *MLL*. Group 1 genes are rich in serine and proline (SP) and contain nuclear localization signals (NLS), and those in group 2 are notable for a cysteine-rich zinc finger (Zn-finger) domain and a leucine-zipper motif. The *AF6* gene contains a novel glycine-leucine-phenylalanine (GLGF) domain, and *AF1P* is distinguished by three acidic (*A*) regions, together with an amino acid repeat motif consisting of aspartic acid–proline-phenylalanine (DPF). Regions on the right of the breakpoints (*arrows*) are retained in the fusion product. (Adapted from Downing JR, Look AT. *MLL* fusion genes in the 11q23 acute leukemias. In: Freireich EJ, Kantarjian H, eds. Leukemia: advances in research and treatment. Boston: Kluwer Academic Publishers, 1995:73–92.)

Leukemic blasts carrying the t(4;11)(q21;q23) tend to have a pro-B phenotype, with expression of HLA-DR antigens and rearranged immunoglobulin heavy-chain genes.[116,117,118 and 119] Clinical correlates of this rearrangement include leukocyte counts more than 100 × $10^9$/L and an adverse prognosis, even with use of intensive combination chemotherapy.[120,121 and 122]

Unlike most other chromosomal lesions in childhood ALL, translocations affecting the 11q23 region can also be found in the myeloid leukemias, suggesting transformation of a pluripotential stem cell. A striking example is the t(9;11)(p22;q23), which occurs with high frequency in acute monocytic leukemia, [123] as well as in mixed-lineage leukemias and myeloid leukemias arising after treatment for ALL. [124] Moreover, leukemic lymphoblasts with 11q23 abnormalities can be induced to express monocytic features *in vitro*, strengthening the notion that such lesions arise in uncommitted stem cells or affect genes that control lineage commitment. [118,119]

Remarkably, the many breakpoints that can occur at the 11q23 locus affect the same gene, regardless of the phenotype of the altered cell. The *MLL* gene, also called *HRX, ALL1*, and *Htrx1*, becomes disrupted within a small region situated midway through the gene's coding region, [125,126,127,128,129 and 130] resulting in loss of two zinc finger regions and retention of an A-T hook domain and a region homologous to mammalian DNA methyltransferase (MT; Fig. 4-7). This consistent pattern of alteration suggests a common mechanism of tumorigenicity. The A-T hooks, which may bind to DNA in the minor groove, could allow interactions with potential target gene promoters and access to other transcription factors, and the MT domain could affect transcription by modifying the methylation status of bound DNA.

Although targets of MLL action have not been identified, it may be important that the normal protein is the mammalian counterpart of trithorax, a putative *Drosophila* transcription factor known to regulate the actions of a wide spectrum of homeotic genes in the bithorax complex of the fly, and it is absolutely required throughout embryogenesis for differentiation of the head, thorax, and abdomen. [126,127,128 and 129,131] The identity of the fusion partner in 11q23 translocations appears to be of secondary importance to retention of the A-T hook and MT domains of the MLL protein, although it may influence the lineage predilection of the leukemia. MLL certainly qualifies as the most promiscuous of all transcription factor proto-oncogenes in acute leukemia, as 23 different partner genes have now been cloned that form fusion proteins with MLL (Table 4-4). Appropriate model systems are needed to explore the mechanistic implications of fusion proteins juxtaposing the same domains of MLL with a variety of partners.

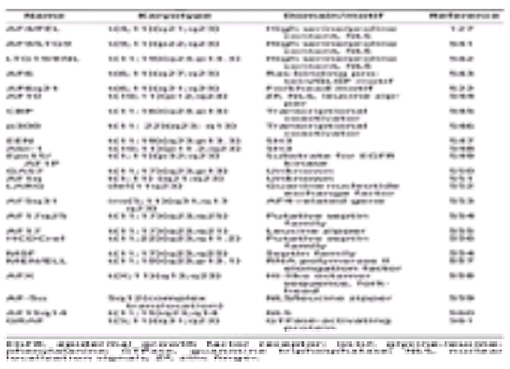


**TABLE 4-4. PARTNER GENES INVOLVED IN MLL REARRANGEMENTS**

The *MLL* gene may participate in leukemogenesis more often than is indicated by studies of 11q23 cytogenetic changes. Schichman and co-workers [132,133] found tandem duplication of an internal portion of the *MLL* gene in the absence of 11q23 translocations, a process that links the intact gene to a duplication of its amino-terminal region. All duplications have contained the A-T hook and DNA MT motifs, reinforcing the presumed critical importance of these regions in leukemia induction.

### *Oncogenic Fusion Genes in Acute Myeloid Leukemia*

The same general mechanisms responsible for proto-oncogene activation in ALL have been identified in AML. A prime example of a chimeric transcription factor in this disease is the AML1-ETO protein resulting from the t(8;21) translocation. [134,135 and 136] The most frequent cytogenetic abnormality in the myeloid leukemias, t(8;21), primarily affects myeloblasts with evidence of granulocytic differentiation [i.e., M2 designation in the French-American-British (FAB) classification scheme]. The sequence-specific DNA-binding and protein-protein interaction properties of the fusion protein are provided by a large domain in the *AML1* gene[137] showing 68% homology with the *Drosophila* pair-rule segmentation gene, runt, whose product helps to determine the structures derived from each segment of the developing embryo.

AML1 forms complexes with members of the CBF protein family, which lack DNA-binding domains.[138,139] One such protein, CBFb, is involved in another major chromosomal rearrangement in AML, the inversion 16, found in association with myelomonocytic differentiation and increased bone marrow eosinophils (i.e., M4-Eo designation in the FAB system). This rearrangement joins the amino-terminal sequences of the *CBFb* gene to the carboxyl terminus of the heavy-chain gene of smooth muscle myosin (*MYH11*), resulting in the formation of a CBFb-MYH11 fusion protein.[140] Both AML1 and CBFb appear to be normally expressed in early myeloid cells, suggesting that the oncogenicity of the respective fusion proteins stems from disruption of a transcriptional regulatory complex specific for myeloid developmental processes.[141]

The combinatorial versatility of the *AML1* locus can be seen from its fusion with sequences from the *EVI1* gene in t(3;21)-positive chronic myeloid leukemia (CML) in blast crisis[142] or the *EAP* gene (coding for Epstein-Barr virus RNA-associated protein) in myelodysplastic syndrome. [143] Inclusion of the runt-homologous DNA binding/dimerization domain of AML1 and the zinc finger DNA-binding domains of EVI1 in the AML1-EVI1 chimeric protein affords numerous opportunities for aberrant regulation of target genes. The AML1-EAP protein contains carboxyl-terminal EAP sequences fused out of frame to AML1 sequences, resulting in a truncated AML1 protein that may dominantly interfere with normal AML1 function during myelopoiesis, as has been postulated for the AML1-ETO chimera. [134]

Leukemia specialists have long envisioned treatments based on a molecular understanding of the proteins that generate and maintain the neoplastic state. Major progress toward this goal has been achieved in acute promyelocytic leukemia (APML) with the t(15;17)(q21;q11-q22), in which the critical ligand- and DNA-binding sequences of the retinoic acid receptor a (*RAR*a) gene on chromosome 17 are fused to sequences of the *PML* gene on chromosome 15.[144,145,146,147 and 148] In its unaltered form, the RARa protein binds first to the retinoic acid ligand and then to DNA through its zinc finger region. PML proteins, which also possess zinc finger motifs, are normally located in novel macromolecular nuclear organelles, called *PML oncogenic domains* (PODs), which include at least three other proteins.[149,150 and 151] The PML-RARa fusion proteins disrupt these subnuclear structures, causing normal PML, RXR, and other nuclear proteins to disperse in an abnormal microparticulate pattern.[149,150 and 151] They also interfere with normal myeloid cell development, possibly through adverse effects on the assembly of PODs that contain PML, leading to arrest of differentiation in the promyelocytic stage.

These fundamental observations provide a rationale for use of all-*trans*-retinoic acid to treat patients with APML.[152,153,154,155 and 156] In pharmacologic doses, the compound binds to the RARa fusion partner, followed by reorganization of PML and its associated proteins into normal-appearing nuclear PODs ( Fig. 4-8). Subsequently, the leukemic cells develop into mature myelocytes with limited life spans. Retinoid treatment of APML does not result in permanent remissions, however. Resistance to the hormone generally develops within 3 to 4 months, limiting the agent's therapeutic role in the remission-induction period and to combination therapy with standard cytotoxic drugs.[136,137,155,156] Nonetheless, the results justify continued efforts to devise therapies directed to the oncogenic proteins that drive neoplastic proliferations in the childhood cancers.

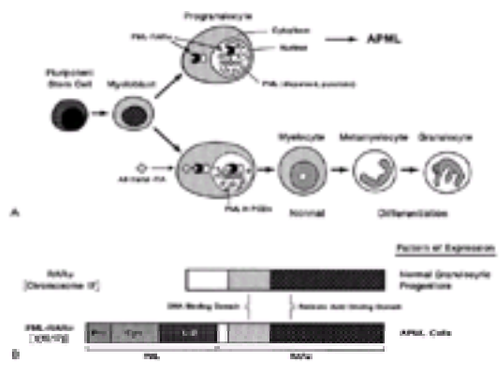



**FIGURE 4-8.** Possible mechanism of action of all-*trans*-retinoic acid in acute promyelocytic leukemia (APML) expressing the PML protein fused to the retinoic acid receptor a protein (PML-RARa). Therapeutic rationales based on knowledge of chimeric transcription factors are rare. In the example shown in **A**, expression of PML-RARa in progranulocytes as a result of the t(15;17) causes dispersion of novel macromolecular nuclear organelles called *PML oncogenic domains* (PODs), leading to the development of APML. Treatment with all-*trans*-retinoic acid (RA) supplies a ligand that binds to the RARa fusion partner, followed by reorganization of PML and its associated proteins in PODs. Subsequently, the leukemic progranulocytes show normal patterns of differentiation. (Adapted from Look AT. Pathobiology of the acute lymphoid leukemia cell. In: Hoffman R, ed. Hematology. 2nd ed. New York: Churchill Livingstone, 1995:1046–1066.) **B:** RARa structural domains retained in the chimeric protein. Pro, proline; Cys, cysteine; L-Z, leucine zipper.

### *MYC Activation in B-Cell Acute Lymphoblastic Leukemia*

ALL often arises from translocations that affect the immunoglobulin and TCR genes of lymphoid progenitors, which must undergo diverse clonal rearrangements to permit development of the specialized populations of T and B cells needed to support a competent immune system. Even though the transformed cells display abnormal growth patterns, they tend to retain the characteristics of normal T and B lymphoblasts. Only rarely does one encounter aberrant gene expression indicating involvement of lymphoid and myeloid cells (i.e., mixed-lineage leukemias). Consequently, the immunophenotypes of developing lymphoid cells correlate remarkably well with particular chromosomal translocations and the transcription factor genes they affect ( Table 4-1). In B-cell acute leukemia (also Burkitt's lymphoma), the predominant (8;14)(q24;q32) translocation moves one allele of MYC, a prototypic bHLH/leucine ipper transcription factor gene on chromosome 8, into the heavy-chain immunoglobulin locus on chromosome 14q32, adjacent to the coding sequences of the immunoglobulin constant region.[157,158 and 159] This juxtaposing of MYC transcriptional promoter sequences with strong immunoglobulin enhancer elements leads to overexpression or inappropriate expression of the MYC protein.

Although the t(8;14) accounts for most B-cell ALL cases with rearranged *MYC* loci, two of its variants are also capable of activating this proto-oncogene. In cells with the t(2;8) or the t(8;22), the *MYC* gene remains on chromosome 8, and portions of the k or l light chain genes on chromosome 2 or 22, respectively, are translocated to a site downstream of the *MYC* locus (reviewed elsewhere[160]). These rearrangements lead to aberrant MYC expression.[161,162,163,164,165 and 166]

How does the MYC oncoprotein transform cells? The favored model posits a transcriptional network involving a minimum of three other factors, each having bHLH and bZIP domains (Fig. 4-9). MYC is able to heterodimerize with the MAX protein,[167,168] which binds to DNA, to itself (i.e., MAX:MAX homodimers), and to other proteins, including MAD and Mxi-1.[169,170] Because only MYC:MAX heterodimers are transcriptionally active, and MYC and MAD have equal affinities for the MAX protein,[171,172] an increase in MYC expression resulting from gene rearrangement could disrupt the MAX heterodimer equilibrium in lymphoid progenitors, leading to inappropriate transcription of downstream targets and ultimately to malignant transformation.[173] Experimental support for this hypothesis comes from the induction of B-cell neoplasms in transgenic mice carrying the MYC oncogene driven by an immunoglobulin gene enhancer.[174,175] An activated MYC gene also induces tumorigenic conversion when it is introduced *in vitro* into B lymphoblasts infected with human Epstein-Barr virus.[176]

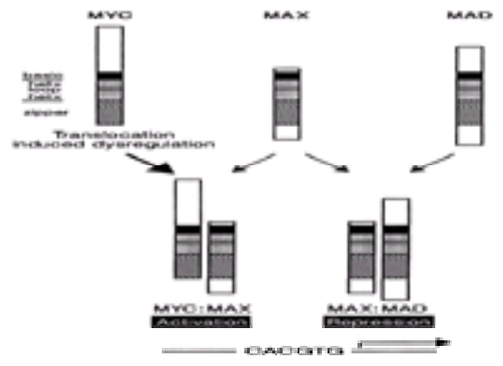



**FIGURE 4-9.** Model for the oncogenicity of the MYC protein activated by the t(8;14) in B-cell acute lymphoblastic leukemia (ALL). Normally, MYC participates in a transcriptional network involving at least two other proteins—MAX and MAD—in which it heterodimerizes with MAX, which can also bind to DNA, to itself, and to MAD. Overexpression of MYC in B-cell ALL is thought to shift the equilibrium between transcriptionally repressive MAX:MAD and activating MYC:MAX heterodimers to the latter complex, leading to aberrant transcription of downstream targets and ultimately to malignant transformation. Conserved DNA-binding and protein dimerization domains are indicated by different patterns of shading. (Adapted from Dowing JR, Look AT. MLL fusion genes in the 11q23 acute leukemias. In: Freireich EJ, Kantarjian H, eds. Leukemia: advances in research and treatment. Boston: Kluwer Academic Publishers, 1995:73–92.)

### *bHLH, LIM, and HOX11 Genes in T-Cell Acute Lymphoblastic Leukemia*

Transcription factor genes are the preferred targets of chromosomal translocations in the acute T-cell leukemias. Notable examples include the bHLH genes *MYC*,[177,178 and 179] *TAL1/SCL*,[180,181 and 182] and *LYL1*,[183] which are essential for the development of other lineages such as erythroid cells (TAL1/SCL), but with the

exception of MYC, they are not expressed in normal T-lymphoid cells. When rearranged near enhancers within the TCR-b–chain locus on chromosome 7, band q34, or the a/d chain locus on chromosome 14, band q11, these regulatory genes become active, and their protein products bind inappropriately to the promoter or enhancer elements of upstream targets.

A useful model of aberrant transcription factor expression in T-cell ALL is provided by *TAL1* activation due to the t(1;14) or to intragenic deletion of the upstream side of the gene, changes that characterize as many as one-fourth of all cases of childhood T-cell leukemias. [184] Because the TAL1 protein dimerizes with the E2A protein to form DNA-binding complexes,[185] its ectopic expression in T cells would be expected to activate specific sets of target genes that are normally quiescent in T-cell progenitors.

Other types of regulatory genes can be rearranged near TCR loci, including those encoding the "LIM domain only" (LMO) proteins, LMO1/RBTN1/TTG1 and LMO1/RBTN2/TTG2, within the cysteine-rich LIM family. [186,187,188 and 189] Although present in high concentrations in the central nervous system, [187] the LMO proteins are only minimally expressed or absent altogether in T cells and their progenitors. Both LMO1 and LMO2 possess zinc finger–like structures in their LIM domains [190] but lack the homeobox DNA-binding domains common to other transcription factors in this family, suggesting that the LIM domain functions in protein-protein rather than protein-DNA interactions. Conceivably, it could even mediate the action of other transcription factors, as indicated by the ability of LMO2 to bind to the bHLH protein TAL1 *in vitro*.[191,192] Moreover, LMO1 induces thymic lymphomas in transgenic mice whose thymocytes bear the *LMO1* gene under the control of a proximal Lck promoter.[193] In this context, inappropriate expression of a LIM family protein appears to have selectively transformed a rare subset of $CD8^{+}$, $CD4^{-}$, and $CD3^{-}$ thymocytes. Whatever the tumorigenic mechanism, ectopic expression of LMO1 or LMO2 because of t(11;14)(p15;q11) or t(11;14)(p13;q11) could be expected to affect similar T-cell developmental pathways.

*HOX11* completes the list of developmental genes that are inappropriately placed under the control of TCR loci. Located on chromosome 10, band q24, [194,195,196 and 197] this gene encodes a homeodomain transcription factor that can bind DNA and transactivate specific target genes. [198] It is most closely related to *Hlx*, a murine homeobox gene expressed in specific hematopoietic cell lineages and during mouse embryogenesis, [199] and it is distantly related to the *antennapedia* homeobox genes of *Drosophila*, which regulate segment-specific gene expression along the anteroposterior axis of the fly embryo. [93] A specific homeotic role of *HOX11* in mammalian development was demonstrated by homozygous disruption of this gene, which blocked the formation of the spleen in otherwise normal mice. [200] In the mouse, Hox11 is normally expressed in specific regions of the branchial arches and ectoderm of the pharyngeal pouches of the developing hindbrain, as well as from a single site corresponding to the splanchnic mesoderm beginning on embryonic day 11.5. [200] Because the nervous system develops normally in these mice, the roles of Hox11 proteins in branchial arch and hindbrain structures appear to be compensated for by other transcription factors expressed by the cells; however, the role of Hox11 in cellular organization at the site of splenic development is absolutely essential for the genesis of this organ. Lymphoid and other types of hematopoietic cells, normally lacking expression of Hox11 proteins, were not affected by loss-of-function mutations in this gene, except for the presence of asplenia-related Howell-Jolly bodies in circulating erythrocytes. Activation of *HOX11* by chromosomal translocations, either the t(10;14)(q24;q11) or the t(7;10)(q35;q24), in developing T cells must therefore interfere with normal regulatory cascades to promote malignant transformation.

### *EVI1 Activation in Acute Myeloid Leukemia*

In some cases of AML with high platelet counts, the inv(3)(q21;q26.2) or the t(3;3)(q21;q26.2) moves promoter/enhancer sequences from one site on chromosome 3 into the *EVI1* locus on the same chromosome,[201] leading to increased gene expression. The same effect is produced in murine myeloid leukemias by insertional mutagenesis.[202] The EVI1 protein binds to promoter/enhancer sequences containing the GATA sequence motif, and its tumorigenicity may come from interference with regulatory signals normally mediated by the GATA family of hematopoietic transcriptional regulators. [203,204,205 and 206] The tissue distribution of EVI1 in oocytes and kidney cells and its dominant interfering effect on normal myelopoiesis suggest an important developmental role in regulatory pathways of proliferation or differentiation.

### *EWS, FUS, and PAX Gene Fusions in Childhood Sarcomas*

Progress in understanding the genetic changes that affect solid tumors has been conspicuously slower than for the acute leukemias, primarily because of the difficulty in obtaining satisfactory karyotypes and in growing solid tumor cells *in vitro*. Nonetheless, chimeric transcription factors arising from chromosomal translocations have been identified in a large number of soft tissue sarcomas (Table 4-2).

The first translocation to be characterized at the molecular level in sarcomas was the t(11;22), which is virtually pathognomonic of Ewing's sarcoma (EWS) or its close relative, primitive neuroectodermal tumor. The fusion gene created by this rearrangement, *EWS-FLI1*,[207] encodes a hybrid protein containing amino-terminal sequences of EWS linked to the Ets-like DNA-binding domain of FLI1 (named for Friend leukemia integration site 1). [208] Using *in vitro* transformation assays, May and colleagues[209] established that malignant conversion of cells by this protein requires both EWS sequences and the FLI1 DNA-binding domain, suggesting that the chimeric protein acts by disrupting transcriptional regulatory pathways.

Variant translocations fuse identical EWS sequences to the DNA-binding domains of two related Ets family members, ERG [210,211,212 and 213] and ETV1 (Table 4-2).[214] As is the case for EWS-FLI1, the RNA recognition motif of EWS is absent from these variant proteins, replaced by the DNA-binding domains of the fusion partners. The amino-terminal sequences of EWS are rich in glutamine, serine, and tyrosine, making this domain a potent *trans*-activator of gene expression.[215,216] Hence, fusion proteins containing the EWS *trans*-activation domain and an Ets-like DNA-binding domain could act by dominantly and aberrantly up-regulating the expression of critical target genes.

Fusion of the *EWS* gene to the bZIP domain of *ATF1* in the wake of a t(12;22)(q13;q12) translocation leads to an entirely different tumor: malignant melanoma of the soft parts.[217] In malignant liposarcoma, the t(12;16)(q13;q14) fuses the amino terminus of an EWS-related gene called *FUS* (or *TLS*) to the bZIP domain of *CHOP*, initially characterized as a non–DNA-binding, dominant negative inhibitor of other bZIP proteins of the CAAT box–binding C/EBP family. [218,219] The abundant EWS and FUS/TLS proteins can form ternary complexes with a variety of RNA-binding proteins, such as A1 and C1/C2. [201] In addition, the amino-terminal sequences of FUS and EWS can be interchanged in chimeric constructs without affecting the results of *trans*-activation and transformation assays.[220]

The ubiquitous involvement of EWS and FUS combined with the lineage-specific association of the DNA-binding domains of fusion proteins in the malignant solid tumors suggests that the DNA-binding region specifies the downstream target gene and thus the phenotype of the arrested and transformed malignant mesenchymal progenitor cell. This principle is well illustrated by the Wilms' tumor gene, *WT1*, which gives rise to desmoplastic round cell tumors when it becomes fused to *EWS*.[221] In its normal state, *WT1* is a potent tumor suppressor gene whose homozygous inactivation leads to Wilms' tumor.

In alveolar rhabdomyosarcoma, translocations affecting chromosome 13q14 fuse either the *PAX3* or the *PAX7* gene with a portion of a forkhead domain gene, *FKHR*.[222,223 and 224] In tumor cells with the t(2;13)(q35;q14), the paired box and homeodomain regions of the *PAX3* gene are preserved, but the carboxyl-terminal sequences are replaced by a portion of the forkhead DNA-binding sequences from *FKHR*. A variant translocation, t(1;13)(p36;q14), truncates the *PAX7* gene in a similar fashion,[224] suggesting that a common set of target genes recognized by both of the PAX proteins are involved in the pathogenesis of rhabdomyosarcoma. The complete or partial constitutional loss of function of the *PAX3* gene has been correlated with a distinctive inherited condition, Waardenburg's syndrome 1, characterized by pigmentary disturbances, lateral displacement of the inner canthus of each eye, deafness, and mental retardation. [225] This observation, together with evidence that partial or complete disruption of the murine *PAX3* gene leads to abnormalities of the central nervous system,[225] suggests that *PAX* genes participate in critical developmental processes and that the PAX3 product must be present in high concentrations at precise junctures during development. The *SYT-SSX* gene of synovial sarcoma remains an enigma because neither fusion element shows homology to previously described sequences. [226]

## Tyrosine Kinase Gene Activation

Cellular tyrosine kinase genes can be aberrantly activated through a variety of mechanisms: truncation of the ligand-binding domain of growth factor receptors, loss or replacement of carboxyl-terminal regulatory tyrosine residues, and point mutations. [227] Instances of childhood tumors with an altered tyrosine kinase gene as the underlying molecular abnormality are rare, although the two leading examples represent substantial groups of patients who present difficult problems in clinical management (Table 4-5).

| Leukemia type | Karyotype | Activated genes | Reference |
|---|---|---|---|
| Ph⁻ CML | t(5;10)(q33;q21.2) | TEL-PDGFβR | 562 |
| Ph⁺ CML, ALL | t(9;22) | BCR-ABL | 563 |
| T-ALL | t(9;12)(p24;p13) | JAK2 | 564,565 |
| AML | t(12;15)(p13;q25) | TRKC/NTRK3 | 566 |
| AML-M3/M4Eo | t(1;12)(q25;p13) | ARG/ABL2 | 524,567 |
| Lymphoma | t(5;6)(q35;p21) | FLT4 | 568 |
| T-ALL | t(1;7)(p34;q34) | LCK | 569,570 |
| Anaplastic large cell lymphoma, non-Hodgkin's lymphomas | t(2;5)(p23;q35) | ALK | 260,571 |
| Myeloproliferative disorder, lymphoma, acute myelogenous leukemia | t(8;13)(p12;q12) | FGFR-1 | 572,573 |

ABL, v-abl Abelson murine leukemia viral oncogene homolog 1; ALK, anaplastic lymphoma kinase; ALL, acute lymphocytic leukemia; ARG, ABL-related gene; CML, chronic myelogenous leukemia; FLT4, fms-related tyrosine kinase 4; JAK2, Janus kinase 2; LCK, lymphocyte-specific protein tyrosine kinase; PGFR-1, fibroblast growth factor receptor 1; PDGFβR, platelet-derived growth factor beta receptor; Ph, Philadelphia chromosome; TRKC/NTRK3, neurotrophic tyrosine kinase, receptor type 3.

**TABLE 4-5. TYROSINE KINASE GENES ALTERED BY CHROMOSOMAL TRANSLOCATIONS IN HEMATOLOGIC MALIGNANCIES**

### *BCR-ABL in the Chronic Myeloid and Lymphoid Leukemias*

The Philadelphia chromosome, a product of the t(9;22)(q34;q11) translocation, was originally identified in patients with CML; subsequently, it was found in 3% to 5% of children and 30% to 40% of adults with ALL.[209,210 and 211,228,229] Its breakpoints on the distal tip of the long arm of chromosome 9 are variable in CML, occurring at any point over a distance of more than 100 kb within the *ABL* proto-oncogene, upstream of the tyrosine kinase domain.[230,231 and 232] By contrast, the breakpoints on chromosome 22 are confined to a 5.8-kb region of DNA known as the *major breakpoint cluster region*,[233] which lies within a gene called *BCR*.[233,234] The 9;22 translocation produces a *BCR-ABL* fusion gene consisting of 58 (upstream) sequences from *BCR* and 3' (downstream) sequences from *ABL*.[235,236,237,238 and 239] The 8.5-kb fusion transcript found in CML encodes a 210-kd hybrid protein that is activated as a tyrosine-specific protein kinase, similar to the *v-abl* protein product.[240,241,242 and 243]

Although routine karyotyping does not distinguish between the t(9;22) in CML and ALL, molecular analysis of the *BCR* and *ABL* proto-oncogenes, which are rearranged in both diseases, has revealed potentially important differences.[244,245 and 246] In ALL, the rearrangement produces a 6.5- to 7.0-kb fusion transcript and a 185- to 190-kd hybrid protein. Both are distinct from the products of the rearranged *BCR-ABL* fusion gene in CML. The breakpoints on chromosome 22 in ALL cases are not within the 5.8-kb region of *BCR* that contains the breakpoints in CML. Instead, they lie in a second minor breakpoint cluster region located farther upstream, within the *BCR* gene.[247,248 and 249] The ALL fusion protein includes amino-terminal BCR amino acids but lacks the internal residues found in the CML fusion proteins near the *BCR-ABL* junction.

The amino-terminal sequences of *ABL* are replaced in oncogenic forms of the gene, by the Moloney virus *gag* gene in the case of *v-abl*[250,251 and 252] and by *BCR* sequences in the *BCR-ABL* fusion gene of CML and ALL.[240,241,242 and 243] Both products of the *v-abl* and the *BCR-ABL* fusion genes can transform pre-B cells, but the latter cannot transform fibroblasts unless it is fused to the *gag* gene product.[253] The amino-terminal alterations influence the ability of *ABL* to function as a lineage-specific transforming gene and affect the tyrosine kinase activity of the protein product.

The exceedingly poor prognosis of patients with the Philadelphia chromosome has been attributed to transformation of a primitive hematopoietic stem cell that is inaccessible to most forms of chemotherapy.[121,229,254,255] Long-term responses (i.e., probable cures) have been induced in a subset of childhood BCR-ABL $^{+}$ ALL patients with low presenting white blood cell counts by using extensively reinforced early phase chemotherapy, followed by rotational treatment with pairs of non–cross-resistant drugs.[256] Maintenance therapy with interferon for patients in complete remission after either chemotherapy or autologous stem cell transplantation may contribute to more stable responses.[257] An experimental drug, STI-571, which inhibits the tyrosine kinase activity of ABL and BCR-ABL has shown a strong antileukemic effect in BCR-ABL$^{+}$ malignancies and will broaden the therapeutic options in the management of this disease.[258] For most patients, however, the recommended strategy is allogeneic bone marrow transplantation in first remission. Patients who lack suitable related donors should be considered candidates for alternative intensified therapies, including unrelated-donor allogeneic bone marrow transplantation in first remission, although relapse rates with such treatment are high and the outcome after relapse is extremely poor.[259]

### *NPM-ALK in Large Cell Lymphoma*

The 2;5 translocation in anaplastic large cell non-Hodgkin's lymphoma creates a novel fusion gene in which amino-terminal sequences from the nucleophosmin (*NPM*) nucleolar phosphoprotein gene on chromosome 5q35 are linked to the catalytic domain from a previously unidentified tyrosine kinase gene on chromosome 2p23, called *ALK* for *anaplastic lymphoma kinase* (Fig. 4-10).[260] Expressed in nervous system–derived cells of small intestine, testis, and brain, but not in normal lymphoid cells, ALK shares greatest homology with members of the insulin receptor subfamily of receptor tyrosine kinases. Unscheduled expression of a truncated ALK kinase in activated T lymphocytes probably contributes to malignant transformation leading to anaplastic large cell lymphoma.

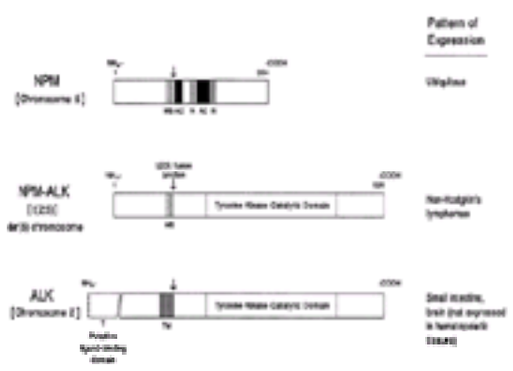



**FIGURE 4-10.** Schematic representation of the NPM and ALK proteins and the fusion resulting from the t(2;5)(p23;q35) in anaplastic large cell non-Hodgkin's lymphoma. Identified in approximately 30% of pediatric large cell lymphomas, the NPM-ALK chimera consists of the N-terminal end of NPM (nucleophosmin) fused in-frame to the intracellular kinase domain of *ALK*, (anaplastic lymphoma kinase). As shown, NPM is a 294–amino acid protein containing a metal-binding domain (MB), acidic amino acid cluster (AC), and two nuclear localization signals (N). The normal transmembrane receptor tyrosine kinase ALK possesses a membrane-spanning region and a kinase domain. The 2;5 translocation results in inappropriate expression of ALK, driven by the strong NPM promoter. Constitutive activation of the protein's kinase activity, potentially through NPM-mediated constitutive dimerization, may contribute to malignant transformation by inappropriately phosphorylating intercellular substrates involved in cell growth or development. [Adapted from Morris SW, Kirstein MN, Valentine MB, et al. Fusion of the tyrosine kinase gene ALK to the nucleolar phosphoprotein gene NPM in human t(2;5)-positive lymphomas. Science 1994;263:281.]

Non-Hodgkin's lymphomas include a diverse group of neoplasms that often present challenging diagnostic problems to the pathologist. In particular, cytogenetic analysis of lymphoma biopsy samples has been difficult in clinical settings, and a significant number of large cell lymphoma cases with the t(2;5) may be overlooked. The ability to reproducibly identify *NPM-ALK* fusion junctions in large cell lymphomas with the t(2;5) using an RNA-based polymerase chain reaction technique should greatly improve early recognition of these tumors.[261]

## Gene Amplification

Gene amplification at the DNA level enables a cell to increase expression of critical genes whose products are ordinarily tightly controlled. The cytogenetic hallmarks

of gene amplification are double-minute chromatin bodies and homogeneously staining regions, which contain the amplified DNA sequences, called *amplicons*.

Several clinically important examples of proto-oncogene amplification can be found among the solid tumors of children and adults. The *MYCN* gene is amplified tenfold to 300-fold in tumor cells from approximately one-third of childhood neuroblastoma cases. Overexpression of the MYCN oncoprotein has been linked to an advanced stage of disease and a poor prognosis.[262,263 and 264] Members of the *MYC* gene family, including *MYC*, *MYCN*, and *MYCL*, are also amplified in DNA extracted from small cell lung cancer lines and show higher levels of amplification when the tumor cells have been exposed to chemotherapy.[265,266] The *HER-2/NEU/ERBB-2* proto-oncogene, a relative of the epidermal growth factor receptor gene, is amplified in approximately one-third of human breast cancers, in which amplified levels of the oncoprotein are associated with poorer disease-free and overall survival rates.[267,268] Extra copies of the *CCND1* (cyclin D1) gene have been reported in breast carcinoma and squamous cell carcinomas of the head and neck,[269,270 and 271] and increased copies of an amplicon on the long arm of chromosome 12, containing the *GLI*, *MDM2*, and *CDK4* genes, have been detected in the sarcomas.[272,273,274 and 275]

Gene amplification has also been reported in isolated cases of human leukemia. For instance, *MYC* was shown to be amplified eightfold to 32-fold in DNA from the HL-60 promyelocytic cell line and in fresh leukemia cells from the same patient.[276,277] The *MYB* gene is amplified in rare cases of AML,[278,279] and the E2F-1 gene is amplified and overexpressed in the HEL human erythroleukemia cell line.[280]

Fluorescence *in situ* hybridization offers a rapid and reliable method for detecting gene amplification in solid tumors.[281,282] Although still in the experimental phase of development, this assay has the potential to improve the staging (hence, prognosis) of several types of tumors, including neuroblastoma and breast carcinoma.[262,263,264,265,266,267 and 268]

### *RAS* and *G-CSF* Gene Activation

Activation of cellular proto-oncogenes by point mutation is difficult to detect because the resulting lesions are not apparent by cytogenetic methods. Through the use of experimental systems, it has been possible to predict the types of genes most likely to be activated by point mutations in human tumors. The *RAS* family comprises the prototypic genes of this class.

Human tumor DNAs were found to contain activated homologues of *HRAS* or *KRAS*[283,284 and 285] after such proto-oncogenes had been identified from comparative studies of viral oncogenes. Gene transfer methods identified an additional *RAS* homologue called *NRAS*[286,287] that had not been previously observed as a component of a transforming retrovirus. These three human genes—*HRAS*, *KRAS*, and *NRAS*—encode 21-kd proteins that are associated with the inner surface of the cytoplasmic membrane[288] and function as intermediates in signal transduction pathways that regulate cell proliferation. The somatic mutations that activate *RAS* proto-oncogenes to transforming status affect the amino acids specified by codons 12, 13, or 61.[289] Mutated *RAS* genes also bind guanine nucleotides, but they have diminished capacity to hydrolyze guanosine triphosphate (GTP) to guanosine diphosphate.[290,291 and 292] The transforming properties of activated RAS proteins may result from their constitutive presence in an activated, GTP-bound form, which continuously activates the RAS signal transduction pathway.

Human *RAS* genes activated by point mutations have shown considerable tumorigenicity in experimental systems, transforming NIH-3T3 murine fibroblasts *in vitro* and primary cultures of embryo fibroblasts.[293,294,295 and 296] Their role in mammalian tumorigenesis is also confirmed by studies of carcinogen-induced animal tumor models.[297,298 and 299]

Among the childhood cancers, activated *NRAS* genes appear to be preferentially involved in the leukemias, having been detected in myeloid cell lines,[300,301] in fresh leukemic cells from patients with acute and CMLs,[302,303 and 304] and in lymphoblastic leukemias with a T-cell immunophenotype.[305] *NRAS* gene mutations involving codon 13 or 61 were found in approximately 20% of AML cases, regardless of the morphologic subtype.[302,306] A mutation of codon 12 of the *KRAS* gene was also observed in 2 of 37 AML cases.[306] In a study of lymphoblasts from children with ALL, cells from 2 of 19 patients showed mutated *NRAS* genes; in both cases, the changes involved codon 12.[307] *RAS* mutations have also been documented in patients with preleukemic syndromes, indicating the potential involvement of these genes in the earliest stages of leukemia development.[308,309]

Point mutations in the gene for the granulocyte colony-stimulating factor receptor in myeloid progenitor cells may also be involved in leukemogenesis. In an analysis of this gene in two cases of myeloid leukemia preceded by severe congenital neutropenia, investigators found a thymine-for-cytosine substitution at the codon for glutamine (i.e., position 718 in one patient and position 731 in the other), leading to truncation of the carboxyl-terminal cytoplasmic region of the receptor.[310] Presumably, activation of the cell cycle and survival-signaling functions of this molecule contributes to the pathogenesis of AML in patients with neutrophil elastase mutations, which disrupt normal myeloid cell differentiation.[311]

## TUMOR SUPPRESSOR GENES

In addition to the proto-oncogene–activating lesions described in the section Proto-Oncogene Conversion, recurring genetic changes in human cells affect a second class of genes, known as *tumor suppressors* or *antioncogenes*, whose products normally provide negative controls of cell proliferation. Loss of function of one or more of these proteins through the deletion or mutational inactivation of their corresponding genes liberates the cell from growth constraints, contributing to malignant transformation. The cumulative effect of genetic lesions that activate proto-oncogenes or inactivate tumor suppressor genes is a breakdown in the balance between cell proliferation and cell loss due to differentiation or apoptosis, resulting in clonal overgrowth within a specific cell lineage. The evidence suggests that the progression of tumors to clinically recognizable forms requires both types of changes.

The first indication that negatively acting proteins contribute to cancer came from research with somatic cell hybrids, showing that tumor cells fused to normal cells no longer exhibited malignant growth properties.[312,313] These observations were supported by the epidemiologic studies of Knudson[7] on the inherited predisposition for retinoblastoma development, characterized by an early age of onset for bilateral tumors compared with a later onset for unilateral tumors. His genetic model of carcinogenesis (Fig. 4-11) specifies two rate-limiting mutations, the first of which can be germline (in bilateral tumors) or somatic (in unilateral tumors), with the second invariably being somatic. Subsequent findings made it clear that both types of inactivating mutations target the alleles of a single susceptibility locus, the *RB* gene, which resides on the long arm of chromosome 13.[10,314]

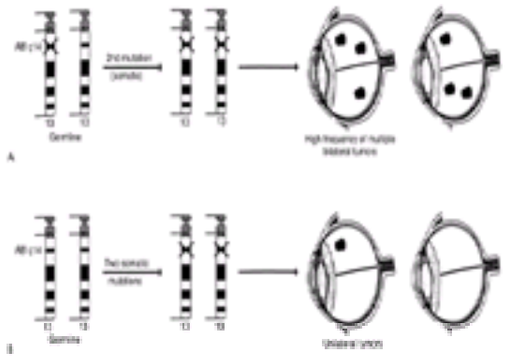


**FIGURE 4-11.** Knudson's hypothesis illustrated by the development of familial **(A)** and sporadic **(B)** retinoblastoma. Children with multiple, bilateral tumors of the eye carry a germline mutation of the *RB* gene that may be inherited or acquired. A somatic mutation affecting the second *RB* allele leads to tumor formation, usually during the first year of life. By contrast, children with single, unilateral tumors developing after infancy lack germline mutations of *RB* but have somatic mutations in both *RB* alleles. The “two-hit” model of carcinogenesis, first proposed by Alfred Knudson, has greatly influenced the direction of cancer research during the past two decades.

Although the list of known and potential tumor suppressors has increased rapidly since the discovery of *RB* (Table 4-4), this gene remains the paradigm for

understanding the dominant mode of inheritance of recessively acting genes that increase susceptibility to cancer. New genes will undoubtedly be added from studies of the hereditary cancer syndromes and from efforts to identify negative regulators of signal transduction pathways. In this chapter, we discuss the *RB*, *p53*, *WT1*, *NF1*, and *NF2* genes and refer briefly to a group of more recently identified suppressors.

### *RB*, the Retinoblastoma Susceptibility Gene

Originally isolated by Friend and co-workers,[11] the *RB* gene was shown to encode a ubiquitously expressed 105- to 110-kd nuclear phosphoprotein, now termed *pRB*.[315,316] The mechanism of pRB tumorigenicity remained elusive until research on the cell cycle revealed the central role of this protein in regulating the progression of cells through the first gap ($G_1$) phase of the cell cycle (as reviewed by Weinberg[317]). As discussed in the section Aberrant Control of the Cell Cycle, pRB serves as a versatile gatekeeper of the cell cycle, transducing physiologic signals that instruct cells to remain in $G_1$ or to move past a defined restriction point and prepare for DNA replication. The transducing function of pRB resides in whether it is phosphorylated by cyclin-kinase complexes. In its unphosphorylated state, pRB binds and represses the function of members of the E2F family of transcription factors, which participate in effector pathways vital to cell division. Phosphorylation results in the uncoupling of pRB and E2F, freeing the latter protein to activate the expression of its downstream target genes.[318]

Any abnormalities that impinge on pRB function could be expected to carry dire consequences, most ominously carcinogenesis. Three classes of viral transforming proteins can deplete cells of pRB by binding to the hypophosphorylated form of the protein.[319,320,321 and 322] The net result is analogous to that produced by inactivation of both *RB* alleles in human retinoblastoma.[320] Most often, the *RB* locus is disrupted by localized deletions of critical gene segments and by point mutations that affect messenger RNA splicing or frameshift mutations leading to truncation of the protein by introducing premature stop codons. Other point mutations give rise to stable proteins with single amino acid substitutions within the binding regions recognized by viral oncoproteins.[323,324 and 325] Such mutants lose the ability to bind cell cycle regulatory proteins, such as E2F, which then can participate without restraint in cell cycle progression (as reviewed by Nevins[318]).

Mutations resulting in homozygous inactivation of the *RB* gene are invariably found in retinoblastoma[316,326,327,328 and 329] but are not restricted to this malignancy. Patients with germline mutations of one *RB* allele characteristically develop bilateral retinoblastoma in the first year of life and are predisposed to develop osteosarcoma and soft tissue sarcomas as well (as reviewed elsewhere[330]), although these tumors occur later in childhood and in adolescence, during the period of maximum growth of these tissues. Somatically acquired *RB* mutations figure prominently in the development of many adult tumors, such as bladder, prostate, breast, cervical, and small cell lung carcinomas,[331,332,333,334,335,336 and 337] even though germline mutations are not predisposing events. Apparently, the inactivation of pRB is an early and essential step in the cascade of genetic changes leading to retinoblastoma and possibly to osteosarcoma; in adult carcinomas, *RB* mutations are not rate limiting but do contribute to tumor progression.

### *p53* Gene

The p53 gene in humans is located on chromosome 17, band p13, and encodes the p53 nuclear protein,[338,339,340 and 341] which is expressed in all cells and tissues of the body. This tumor suppressor has one of the most important etiologic roles in human cancer. It can be inactivated by mutation or lost through chromosomal deletion in a wide variety of human tumors,[342] including carcinomas of the colon,[343,344] lung,[345] breast,[346] esophagus,[347,348] stomach,[349] liver,[350,351] anus,[352] ovary,[353] and prostate.[354] In these neoplasms, *p53* mutations tend to occur as point mutations within the gene's four highly evolutionarily conserved domains, producing a protein that lacks normal regulatory function. Often, the second allele is lost from the malignant clone by deletion, resulting in a reduction to homozygosity for the mutant allele.[342,343] The *p53* gene is also frequently inactivated in childhood tumors such as osteosarcoma,[355,356 and 357] rhabdomyosarcoma,[357,358] brain tumors,[342] CML in blast crisis,[359] Burkitt's lymphoma, and B-cell leukemia, and less frequently inactivated in T-cell– or B-cell–progenitor ALL.[360,361 and 362] Although these tumors can arise from the same types of missense mutations that occur in carcinomas, more often they contain deletions or gross chromosomal rearrangements of both alleles, resulting in total loss of the p53 protein rather than in the production of a faulty protein.

Heritable cancer-associated changes of the *p53* tumor suppressor gene occur in families with Li-Fraumeni syndrome, an autosomal dominant predisposition for the development of rhabdomyosarcoma, other soft tissue and bone sarcomas, premenopausal breast cancer, brain tumors, adrenocortical cell carcinoma, and acute leukemia.[363,364,365 and 366] The Li-Fraumeni syndrome appears to be rare, consistent with its autosomal dominant pattern of inheritance and its high fatality rate.[367] Statistical modeling suggests a 50% probability of invasive cancer by age 30 years in members of Li-Fraumeni families who carry the mutated *p53* gene, compared with a 1% risk by age 30 years in the general population.[367,368] Germinal *p53* mutations could be inherited or could arise as de novo mutations early in embryogenesis or in one of the parent's germ cells.

In contrast to patients with bilateral retinoblastoma, in whom 85% of germline mutations in the *RB* gene appear to arise de novo,[7,11] most germline mutations of the *p53* gene are inherited from an affected parent. This principle became clear from detection of inherited *p53* mutations in children and young adults who developed second malignant neoplasms after successful treatment for the first cancers but who lacked a typical family history of cancer.[342,364] Most inherited mutations of the *p53* tumor suppressor gene produce functionless proteins,[366,369,370 and 371] although splice-site mutations that affect conserved intron-exon boundary sequences of the *p53* gene, resulting in frameshifts and premature termination signals, may account for a significant fraction of the Li-Fraumeni syndrome families in whom no exonic missense mutation has been found.[372,373] These findings suggest the need to examine the entire *p53* gene for splice-site, frameshift, nonsense, and missense mutations in families with multiple cancers of the types found in the Li-Fraumeni syndrome. Because epidemiologic criteria alone are imprecise and can lead to confusion between familial cancer syndromes caused by mutations in tumor suppressor genes other than *p53*,[374,375 and 376] the term p53 *familial cancer syndrome* should be applied to clusters of tumors in families with documented germline *p53* mutations, regardless of the histopathologic findings or pattern of tumor development.[372]

Diagnosis of the *p53* familial cancer syndrome depends on molecular analysis of the entire *p53* coding and splice-site consensus sequences in noncancerous tissue, a technical feat achievable with improved screening methods, such as constant denaturant gel electrophoresis,[377] single-strand conformational polymorphism analysis,[378] functional screens in yeast recombination assays,[379,380] or RNA-DNA heteroduplex digestion.[381] Identification of childhood cancer patients who carry germline mutations of the *p53* gene has had a significant impact on our understanding of the origins of pediatric tumors and will increasingly influence our approach to therapy and genetic counseling of affected persons.[382] The impact of genetic screening will also extend to family members who harbor a mutated allele of the *p53* gene. Knowledge of *p53* gene status could influence reproductive decisions, guide health screening, and lead to studies of prophylactic measures to prevent tumors in affected family members who have not developed malignant disease.

The existence of multiple independent *p53* mutations in both sporadic and familial tumors,[369,383,384] and the variety of familial tumors that can result from a single point mutation[369,384] suggest that the wild-type p53 gene product is critical to normal cellular DNA damage-response pathways and that alterations in *p53* function lead to the transformation of diverse cell types. Available evidence suggests that *p53* functions, at least in part, as a transcription factor[384,385 and 386] and that its conserved domains include an amino-terminal activation domain[387,388] and a central sequence-specific DNA-binding domain.[389,390] Clues to the types of genes targeted by *p53* came from the realization that its levels are increased in response to DNA damage by ultraviolet radiation.[391] The prevailing view is that *p53* functions as a cell cycle checkpoint, blocking cell division in $G_1$ phase to allow repair of damaged DNA or even triggering apoptosis in cells that have defective genomes.[392] In this way, the protein is thought to function almost exclusively as a tumor suppressor, preventing the development of malignant clones from cells with damaged genomes.

This view is borne out by the phenotype of mice that have been rendered p53 deficient by homologous recombination. A total absence of p53 protein expression in the mouse does not produce deleterious effects during embryologic development, but it predisposes these animals to the early development of a variety of neoplasms.[370,393] Thus, the p53 protein is not essential for normal cell division within any lineage during development; rather it acts as a gatekeeper, stopping the cell cycle and repairing or removing cells with damaged genomes that might otherwise evolve into malignant tumors.

### *WT1*, the Wilms' Tumor Gene

The *WT1* gene, located on chromosome 11, band p13, encodes a 50-kd nuclear transcription factor that contains four zinc finger DNA-binding domains (reviewed elsewhere[394,395]). Its identification was the logical end of studies demonstrating large constitutional deletions of the 11p13 region in patients who developed the WAGR syndrome.[13] As predicted by Knudson's hypothesis, tumors from these patients showed mutations of WT1 in the remaining allele, indicating homozygous loss at this locus.[396] Somatic disruption of both WT1 alleles has also been demonstrated in patients with unilateral Wilms' tumors.[397] Overall, 5% to 10% of Wilms' tumors have demonstrable homozygous WT1 mutations,[397,398] although this number may be underestimated, because approximately 20% of such tumors show a loss of

heterozygosity within the region encompassing this gene.[14,15,399]

Unique *WT1* mutations have been demonstrated in individuals with Denys-Drash syndrome, which includes intersexual disorders, nephropathy (e.g., mesangial sclerosis), and Wilms' tumor. These constitutional missense mutations affect single amino acid residues within exon 9 of the gene, which encodes the third zinc finger of the protein.[400,401,402,403 and 404] A defective WT1 protein produced from one allele can apparently interfere with the function of normal WT1 produced from the intact allele, producing developmental anomalies and tumor predisposition. An additional form of the protein resulting from a splicing alteration and lacking exon 2 has been identified in tumor cells but not normal cells, and this combination may represent a distinct mechanism for inactivation of *WT1* in Wilms' tumor.[405]

Expression of the *WT1* gene is restricted during development to mesenchymal tissues, occurring in specific cells of the collecting system within the kidney; non–germ cell components of the gonads, uterus, and spleen; and the mesothelium.[14,16,406] Targeted disruptions of the *wt1* gene in the mouse result in embryonic lethality in the homozygous state, secondary to the failure of kidney and gonad development.[407] The transcriptional properties of the WT1 protein have been analyzed extensively *in vitro*, and it has been shown to be a repressor of several genes encoding proteins important in cell growth control, including insulin-like growth factor II, platelet-derived growth factor, and epidermal growth factor 1.[408,409 and 410] Evidence suggests that the WT1 protein can also activate transcription in certain promoter contexts.[411] The precise mechanism by which WT1 exerts its effects during normal development and prevents tumor formation in the kidney awaits identification of the actual target genes that it regulates *in vivo*.

**Neurofibromatosis Genes, *NF1* and *NF2***

*NF1* is constitutionally mutated in von Recklinghausen's (type 1) neurofibromatosis, an inherited condition characterized by abnormal proliferation of cells of neural crest origin (reviewed elsewhere[412]). In addition to benign neurofibromas, patients with type 1 neurofibromatosis are at increased risk for developing malignant tumors such as pheochromocytomas and malignant schwannomas, which involve loss of the normal *NF1* allele, consistent with the interpretation that *NF1* acts as a tumor suppressor gene in cells of neural crest origin.[413,414 and 415] Another report linked loss of the normal *NF1* allele to malignant myeloid disorders, such as juvenile chronic myelogenous leukemia, which occur with increased frequency in neurofibromatosis patients, suggesting that NF1 may participate in the down-regulation of RAS proteins early in myelopoiesis.[416] Reports have also documented loss of expression and somatic deletion of *NF1* in neuroblastoma and melanoma,[417,418] even though the frequency of these tumors is not increased in neurofibromatosis patients. Thus, NF1 mutations can be an early and predisposing event in the progression of some tumor types but a later and non–rate-limiting step in others.

NF1 is related to GTPase-activating protein, which catalyzes the hydrolysis of the activated GTP-bound form of RAS proteins. Loss of NF1 appears to down-regulate GTPase activity in schwannomas, consistent with a mechanism involving constitutive activation of the RAS pathway, but neuroblastomas and melanomas lacking NF1 have normal GTPase activity, suggesting that loss of NF1 function can contribute to tumorigenesis through a separate and unknown mechanism.[417,418]

The spectrum of malignancies associated with neurofibromatosis type 2 include astrocytomas, meningiomas, and melanomas. The gene associated with this condition, *NF2*, is found on the long arm of chromosome 22 and encodes a protein of the merlin (i.e., moesin, ezrin, radixin-like; also known as *schwannomin*) family believed to be involved in a linkage of the cellular cytoskeleton to the cellular membrane and with cell-cell and cell-extracellular matrix interactions.[419,420 and 421] The mutations in *NF2* result in the formation of a truncated protein. Sporadic meningiomas commonly are associated with mutations of the *NF2* gene, and loss of the chromosome 22 carrying the normal *NF2* gene often accompanies the development of meningiomas.

**Other Known Tumor Suppressors and Newly Identified Candidates**

*RB*, *p53*, *WT1*, *NF1*, and *NF2* are just 5 of the 12 tumor suppressor genes that have been molecularly cloned at the time of writing (Table 4-6). The others—von Hippel-Lindau (*VHL*),[422] adenomatous polyposis coli (*APC*),[423,424] deleted in colorectal cancer (*DCC*),[425] inhibitor of CDK4 ($p16^{INK4A}$, reviewed elsewhere[426,427]), ataxia-telangiectasia mutated (*ATM*),[428] the PITSLRE kinase locus (*CDC2L1*),[429] and breast cancer 1 (*BRCA1*)[430]—were discovered more recently, and less is known about their mechanisms of action. However, some of these proteins have shown a remarkable ability to control signal transduction pathways by forming complexes with other regulators of cell growth and development. For example, the VHL tumor suppressor was found to bind to the heterotrimeric elongin (SIII) complex, thereby inhibiting its capacity to regulate transcriptional elongation of messenger RNA by RNA polymerase II.[431,432,433 and 434]

**TABLE 4-6. MOLECULARLY CLONED TUMOR SUPPRESSOR GENES AND THEIR FUNCTION IN PATHOGENESIS OF NEOPLASMS**

Linkage studies and identification of consistent chromosomal deletions will undoubtedly add new tumor suppressor candidates to the growing list. An especially rich source may be the protein inhibitors of cyclin-dependent kinases (CDKs), which positively regulate the $G_1$ phase of the cell cycle. It is already clear that two specific inhibitors of the cyclin D–associated kinases, $p16^{INK4A}$ and $p15^{INK4B}$, are frequently deleted in a wide variety of leukemias and solid tumors,[427] suggesting that the removal of negative cell cycle controls may be an important step in tumorigenesis.

## ABERRANT CONTROL OF THE CELL CYCLE

Early studies with the light microscope depicted eukaryotic cell division as a mysterious process in which unknown factors held the chromosomes in proper alignment, dictated their duplication, and then signaled for their condensation and segregation. This view has given way to the realization that orderly cell division requires a complex set of molecular controls, coordinated in part by the CDKs.[435,436 and 437] Many of the signals that drive cells from one phase of the cell cycle to another culminate in the phosphorylation of proteins by CDKs, whose active forms are complexes of a catalytic kinase subunit and a regulatory cyclin subunit and often other proteins. Changes in the components of cyclin-kinase complexes determine the proteins that are activated or inactivated by phosphorylation and hence ensure the integrity of the cell cycle. In mammalian cells, the series of kinase and cyclin subunits that are expressed during progression from the first gap phase ($G_1$) to mitosis have been designated CDKs 1 through 8 and cyclins A through H.[435,436 and 437]

Cells must also depend on other kinases, phosphatases, and inhibitors to regulate the CDKs. Perhaps the most intriguing of these factors are the CDK inhibitors (CDKIs)—$p15^{INK4B}$, $p16^{INK4A}$, $p18^{INK4C}$, $p19^{INK4D}$, $p21^{CIP1,WAF1,SDI1,CAP20}$, $p27^{KIP1}$, and $p57^{KIP2}$—which negatively regulate the cell cycle in response to internal and external stimuli.[426] The product of the retinoblastoma tumor suppressor gene, pRB, occupies a central position in the interplay among several of the cyclins, CDKs, and CDKIs (Fig. 4-12).[317] In mammalian cells, this protein undergoes phosphorylation at a point approximately two-thirds of the way through $G_1$ phase, a time coinciding with the cells' advance through a so-called restriction point (R), marked by a transition from mitogen dependence to relative independence from serum factors.[438]

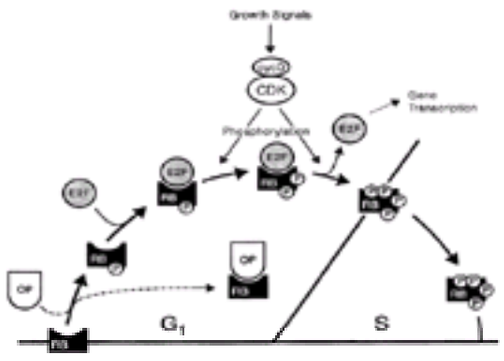



**FIGURE 4-12.** Multifaceted role of the retinoblastoma susceptibility gene product (pRB) in regulation of the $G_1$ to S cell cycle transition. As a nonphosphorylated or hypophosphorylated protein entering $G_1$ phase, pRB binds the E2F transcription factor or a related protein. Because of the action of cyclin D-CDK kinase complexes (cycD-CDK) in response to growth signals originating outside the cell, pRB becomes hyperphosphorylated and releases E2F to activate a group of genes whose products participate in critical downstream effector pathways. Growth inhibitory signals block pRB phosphorylation and thus the passage of cells through $G_1$. Alternatively, some viral oncoproteins (OP) can bind hypophosphorylated pRB, making it unavailable for interaction with E2F ( *dashed line*), so that the cell cycle proceeds without the usual restraints. Mutations in *RB* can likewise prevent pRB-E2F binding, resulting in dysregulated cell proliferation.

Considerable evidence indicates that pRB phosphorylation by CDKs (i.e., CDK4, CDK6, and possibly CDK2), under regulation of the D cyclins, determines whether a cell can successfully breach the R point and be eligible to reach the DNA synthetic (S) phase. Current models uggest that growth signals originating outside the cell stimulate pRB phosphorylation largely through increases in cyclin D levels, leading to activation of CDK4 and CDK6, and perhaps through extended kinase cascades.[426] In its phosphorylated form, pRB uncouples from an E2F family partner, enabling the transcription factor to activate a number of genes (e.g., *MYC*) whose products are important components of downstream effector pathways (reviewed elsewhere[318]). Growth inhibitory signals emanating from outside the cell can block pRB phosphorylation and hence the passage of cells through $G_1$.[317] The retinoblastoma gene product acts as a restriction-point turnstile, transducing physiologic signals into “go–no go” decisions at the critical $G_1$ to S phase transition.

Because cancer cells show many defects in proliferation, one would predict a carcinogenic role for the proteins affecting pRB function. Evidence to support this notion is quite compelling. Mechanisms causing inactivation of pRB include mutations of the *RB* gene that abolish pRB function in retinoblastoma, sarcomas, and other tumors.[335] Other mechanisms operate through increased phosphorylation, rendering pRB inactive, including overexpression of the cyclin D1 gene ( *PRAD1*) as a result of rearrangement with the parathyroid hormone gene locus in benign parathyroid adenomas [439] and cyclin D1 amplification and overexpression in approximately 20% of breast carcinoma cases,[440] 34% to 64% of head and neck squamous cell carcinomas,[441,442] and [443] 30% of esophageal cancers,[444] and 10% of hepatocellular carcinomas.[445,446] Similar increases in pRB phosphorylation may result from amplification of the *CDK4* gene, a common finding in many glioblastomas[447,448] and sarcomas.[272,273,274] and [275]

One of the most striking observations is the frequent deletion of the $p16^{INK4A}$ and $p15^{INK4B}$ CDKI genes from chromosome 9p21 in cases of childhood ALL with either a T or B immunophenotype.[427,449] These tandemly linked *CDKI* genes function as specific inhibitors of cyclin D–associated kinases, suggesting that their elimination within cells could remove negative growth regulatory signals emanating from pRB, resulting in a proliferative advantage and perhaps tumor formation under some conditions. The implications of this discovery are far reaching, in that the 9p21 band, where the $p16^{INK4A}$/$p15^{INK4B}$ locus resides, is a frequent target of homozygous inactivation by deletion and mutation. From the studies reported, $p16^{INK4A}$ appears to be one of the most frequently altered components of the cell cycle machinery in human cancer.[427] It should be stressed that the cancer-related molecular changes described previously ultimately involve pRB inactivation, by which cells are given unrestrained passage into late $G_1$ phase and subsequently are able to progress unimpeded into S phase, the period of DNA replication.

Breakdowns in the p53 checkpoint constitute a second major class of carcinogenic events linked to the cell cycle. [392] If cells become genetically damaged while in $G_2$, M, or early to middle $G_1$, there is an increase in steady state levels of p53, leading to activation of the p21 CDK inhibitor, which blocks the activity of CDK complexes—including those that normally phosphorylate pRB—and prevents DNA replication by binding to PCNA. [450,451,452,453,454] and [455] In this manner, defective cells are “gated” from further progression through the cell cycle while DNA repair enzymes restore the integrity of the genome. This mechanism is fundamental to preventing inadvertent replication of unrepaired DNA sequences that could be passed on to succeeding generations of progeny. When the p53 checkpoint is not available to cells because of inactivating mutations or gene deletions, conditions favor a greatly increased rate of cancer induction. [392]

Whether inappropriately expressed cell cycle control genes serve as true oncogenes is unclear. Their transforming ability in experimental systems has not been impressive, generally requiring additional genetic events to produce frank neoplastic changes. [427] The most appealing hypothesis is that defects in cell cycle control are not in themselves sufficient to cause malignancy. Rather, they give rise to uncontrolled proliferation and genetic instability preceding and predisposing to chromosomal rearrangements, gene amplifications, and aneuploidy—the hallmarks of clinically apparent neoplasia. They may constitute only a single step in the complex series of changes that produce a pediatric cancer.

Molecular lesions in the cell cycle machinery may ultimately afford a new kind of target for chemotherapy. [456,457] For example, much of the success of modern chemotherapy against the childhood leukemias can be attributed to the rapid induction of apoptosis, but in other types of cancer, the cells appear relatively resistant to this effect. Because some of the gene products that control progression from $G_1$ to S phase (e.g., p53) are also involved in apoptosis, [458,459,460] and [461] it may be possible to target this checkpoint so that malignant cells would be directed to apoptosis instead of cycle arrest. Other potential therapeutic targets include proteins involved in the choreographed degradation of cyclin/CDK complexes, which must be removed after they are needed for the cell cycle to continue (reviewed elsewhere[462]). As our knowledge of cell cycle control elements increases, it should be possible to devise therapeutic strategies that take advantage of differences in cycle regulation between normal and malignant cells.

## DEFECTS IN MISMATCH RECOGNITION AND REPAIR

Living organisms have been selected for their ability to replicate their genomes with fidelity but not absolute precision. This property ensures a certain degree of species continuity and stability while allowing the dynamic of genetic change essential to environmental adaptation and consequent evolution. The process of DNA replication, so fundamental to our understanding of biology and inheritance, has been a focus of investigation from the beginning of the modern era of biologic research. The unwinding and copying enzymes that replicate DNA on the basis of complementarity of nucleotides (i.e., guanosine with cytidine and adenosine with thymidine) and hydrogen bond formation form a highly efficient and accurate replicative complex. However, the process of DNA replication is not perfect.

Mistakes in base pairing are occasionally made depending on the organism, the specificity and accuracy of individual DNA polymerases, and the peculiarities of the local environment that may make such mistakes more or less likely. Some stretches of DNA are more likely to accumulate errors than others. In particular, stretches of DNA that consist of tandemly repeated units can present difficulties to precise replication by DNA polymerases. Such mono ($[A]_n$), di ($[CA]_n$), tri ($[CAG]_n$), or tetra ($[CACG]_n$) nucleotide repeats can lead to *strand slippage* during the replication process, in which the two DNA strands come apart and then reanneal with appropriate hydrogen bond formation but out of register by one or more repeat units ( Fig. 4-13). These areas of tandem repeats are also called *microsatellite regions.* Assaying their frequency of gain or loss of one or more repeat units (i.e., their relative instability) has become a means of determining the mismatch repair capability of a particular cell line, tumor, or tissue.

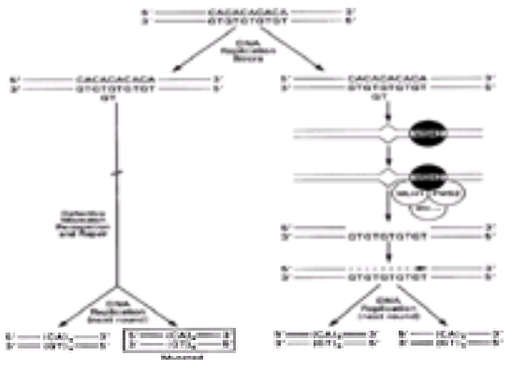


**FIGURE 4-13.** Competent or defective nucleotide mismatch recognition and repair after “strand slippage” during DNA replication. During DNA replication, a looping out of one of the “microsatellite” repeat units occurs on one or another strand. In the example on the right of the figure, this looped-out sequence is recognized as a mismatch. The recently synthesized strand is targeted for excision, partial resynthesis, and ligation by the mismatch repair complex consisting of MSH2, MLH1, PMS2, and other enzymes. The result is the maintenance of sequence fidelity. On the left of the figure, in the absence of a competent mismatch recognition and repair complex, the looped-out nucleotides are not dealt with. At the next round of DNA replication, one of the two daughter cells receives (in this example) an allele for this sequence in which an additional repeat unit has been added to the genomic sequence.

Certain types of cancers show marked defectiveness in their ability to recognize and repair nucleotide mismatches and thereby have marked instability in the microsatellite repeats scattered throughout their genomes, called *replication errors*. For these malignancies, it is believed that the mismatch repair defectiveness is an early step in the process leading to their malignant transformation. [463,464] The consequence of this defect is hypermutability within the cell. Such hypermutability creates a situation in which mutations of growth-affecting genes become much more likely. [465]

Positive selection of the hypermutable, growth-dysregulated clone leads incrementally to malignant transformation. The genes that are responsible for recognition and repair of nucleotide mismatches have been conserved in evolution from bacteria to humans. [466] Of what is likely to be a larger number, six human genes have been identified that play distinct roles in nucleotide mismatch recognition and repair: *MSH2*,[467,468] *MLH1*,[469,470] *PMS1*,[471] *PMS2*,[471] *GTBP* (MSH6),[472,473] and [474] and *MSH3*.[475,476]

**Hereditary Nonpolyposis Colorectal Cancer**

The etiologic impact of defective nucleotide mismatch repair in oncogenesis is most firmly established for a subgroup of colorectal cancers. Within this subgroup is included the hereditary predisposition to colorectal cancer, a condition called *hereditary nonpolyposis colorectal cancer* (HNPCC) or Lynch's syndrome.[477] Polyps are not absent in the families affected by this syndrome, but the clinical manifestations are distinct compared with another and less common form of hereditary predisposition to colon cancer, familial adenomatous polyposis, in which the affected individuals manifest hundreds to thousands of colonic polyps at an early age and, at the molecular level, are found to carry a germline mutation of the *APC* gene.[478]

HNPCC probably accounts for approximately 5% of all colorectal cancer cases. The actual percentage is still controversial, because most estimates are based on family studies or information from referral centers, and definitive population-based studies are still in progress. General criteria for defining HNPCC have been suggested by the International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer. [479] These include the onset of colorectal cancer in at least three individuals spanning two generations, at least one of whom is a first-degree relative of the other two, with the diagnosis for at least one of the individuals occurring before age 50 years. The stringency of these criteria makes it likely that a number of individuals with an inherited predisposition to colorectal cancer may not be included in this defined set. A higher proportion of HNPCC malignancies occur in the cecum and ascending colon than in other colorectal sites, where tumor development is based on different genetic mutations. The pathology of colorectal cancer in these families is not entirely distinctive but does include an increase in the number of tumors that are poorly differentiated and produce mucin.[477] Such pathologic findings are often seen in children with colorectal cancer as well. Families with HNPCC also show an increased susceptibility to some other kinds of cancer. In particular, there have been associations with endometrial and ovarian malignancies in women and some reports of an increased predisposition to stomach, pancreatic, and genitourinary cancers. [480]

It is unclear what determines the sensitivity or resistance to malignant transformation of particular cell lineages affected by defective mismatch repair. The distinctive pattern of malignancies for which an HNPCC gene carrier is at risk suggests that some kind of environmental or hormonal influence makes certain tissues with defective mismatch repair more susceptible to malignant transformation, or that regions of the genome more susceptible to the effects of a failure in nucleotide mismatch correction are regions that influence cell growth in only a subset of cell lineages.

The propensity to develop nucleotide mismatches during normal cell growth and the conservation of the mismatch repair system throughout evolution suggest that this mechanism is critical to normal development. However, it also seems that a defective mismatch repair system is not incompatible with viability. In general, the nontumor tissue of patients with HNPCC have normal microsatellite stability, suggesting that a single normal allele of the relevant genes is all that is needed for appropriate nucleotide mismatch recognition and repair. In these HNPCC patients, only their tumors, having experienced a loss of the other normal allele, show microsatellite instability. In some individuals, however, mutations in *MLH1* or *PMS2* apparently result in microsatellite instability in all tissues of the body. [481] These persons do not differ appreciably from HNPCC patients whose nontumor tissue appears normal. They do not experience a marked increase in cancer above the already increased predisposition imposed by HNPCC itself, although one could interpret the data as suggesting that the onset of colorectal cancer might be occurring earlier than in other HNPCC kindreds (i.e., an individual had the onset of colorectal cancer at age 12 years and another had two separate colorectal cancers by age 31). The spectrum of malignancies, however, is not significantly expanded.

Murine models have been developed in which *MSH2*, *MLH1*, or *PMS2* was homozygously deleted.[482,483] and [484] Such mice are viable, although in the case of the *PMS2* deletion, the males are infertile, with spermatozoa showing aberrant synapsis during the first meiotic division and in the *MLH1*-deleted mouse both males and females are sterile. In such mice, the spectrum of malignancy does seem to be expanded to include hematopoietic neoplasms, particularly lymphomas.

The spectrum of malignancies influenced by defective nucleotide mismatch recognition and repair has been extended into the pediatric population. In Turcot's syndrome, some of the associated colorectal adenomas and primary central nervous system tumors can manifest microsatellite instability. [485,486] Mutations of *MLH1* or *PMS2* were demonstrated in association with colorectal adenomas and glioblastoma multiforme in 2 of 14 registry-defined Turcot's syndrome families; ten of the families did not show defective mismatch repair but did carry germline mutations of the *APC* gene. The predominant brain tumor in these ten families was medulloblastoma.

**Sporadic Colorectal Cancer**

Defective nucleotide mismatch recognition and repair are etiologic factors in sporadic malignancies. It is estimated that 10% to 20% of the colorectal cancers occurring in individuals without a significant family history demonstrate microsatellite instability, compared with nonmalignant tissue from the same person. In the sporadic colorectal cancers that do show microsatellite instability, it is often impossible to implicate a mutation in *MSH2*, *MLH1*, *PMS1*, *PMS2*, or *GTBP* as the basis for the mismatch repair defect. Approximately one-half of the sporadic mismatch repair–defective colorectal cancers carry biallelic mutations of one of these genes in the tumor cells.

The frequency of colorectal cancers with defective mismatch repair is higher in younger adults with an inherited predisposition to cancer than in older persons. [487,488] The incidence of mismatch repair–defective tumors, believed to arise on the basis of somatic mutation, begins to rise again in the elderly. [489] In the older group, fewer than 10% of the individuals harboring these tumors carry a germline mutation in one of five critical genes, contrasted with 40% to 50% of patients younger than 35 years.[489] This younger population comprises a mixture of individuals from previously unrecognized HNPCC families and others who carry de novo germline mutations. There can be variable penetrance of colorectal cancer in families who carry the same gene mutation, [489] suggesting that other genetic and environmental factors influence whether a mismatch repair mutation progresses to overt cancer.

### Targets of Defective Nucleotide Mismatch Repair

A cell that is defective in the recognition and repair of nucleotide mismatches is not, based only on this defect, a malignant cell. The defect predisposes to the development in certain cell lineages of additional oncogenic mutations, which then combine to malignantly transform the cell, giving rise to a cancer. Oncogenic targets for defective nucleotide mismatch repair, therefore, are of interest for charting the pathway from a normal cell to cancer.

One such target in mismatch repair–defective colorectal cancer may be one of the two polypeptides that serve as the receptor for the growth-controlling factor: transforming growth factor-b (TGF-b). Within the coding region of one of the chains of the TGF-b receptor (TGF-b RII) is a run of ten adenosines. It had previously been demonstrated that certain colorectal adenoma cell lines became resistant to the growth-controlling effect of exogenously administered TGF-b as they progressed to frank malignancy.[490] This finding was extended, and resistance to growth control by TGF-b was found to occur exclusively in the subgroup of colorectal malignancies that were defective in nucleotide mismatch recognition and repair. A closer look at the receptor for TGF-b in these tumors showed that they no longer made functional TGF-b RII polypeptide because of frameshift mutations within the track of ten adenosines found within the coding sequence. [491] The frameshifts involved gain or loss of A residues, consistent with the types of mutations associated with microsatellite instability. At least one possible consequence of mismatch repair defectiveness is the increased propensity to mutate the TGF-b RII molecule, enabling cells to escape from the antiproliferative effects of TGF-b.

## TELOMERASE

Aging is a complex phenomenon. In a textbook on pediatric oncology, it is perhaps puzzling to encounter a discussion that begins with a sentence conveying a common geriatric focus. However, it is worth considering through certain examples that there are common features within the fundamental themes of development, aging, and malignant transformation. Much of what is considered to be aging (and dying) can be related to how the lifestyles, behavior patterns, and genetic predispositions of individuals interact to determine physiologic responses to DNA damage and metabolic and toxic challenges.

Although there has been a marked increase in the average life expectancy of humans in developed countries during the past century, there is no evidence that even the most carefully ingested diet and well-structured exercise program delivered within the most pristine and germ-free environment could result in immortality or even a substantial lengthening of life beyond the 100 to 120 years that appear to be the maximum life expectancy for *Homo sapiens*. One possibility is that even in the most controlled of environments, the accumulation of mutations in critical cells and tissues eventually leads to organ failure and death. Although the effect of accumulated mutations should not be discounted, it is also clear that individuals and their cells are programmed from birth to have a finite lifespan. It is only cancers that have been able to circumvent this program and achieve a kind of immortality.

Cells derived from the explants of tissues from higher organisms grow in culture for a limited time that can be measured in cell *generations*. After a certain number of doublings, often characteristic of the animal from which the cells were derived, the culture goes into a crisis and then dies. An inherent “biologic clock” seems to govern cell senescence. The process of cell death may be a passive process in which the nutrient and metabolic resources of the cell deteriorate or an active process in which, in response to senescent stress, a group of death genes are expressed that lead the cell to commit suicide.

What is the basis of the clock whose ticking proscribes our immortality? One potential mechanism in eukaryotes is directly related to the linear structure of eukaryotic chromosomes; they have ends, called *telomeres*.

### Telomeres

Telomeres have a specific structure characterized by dozens to thousands of simple G-rich repeats (distinct for distinct species; the human telomere repeat is TTAGGG) that run 58 to 38 toward the end of the chromosome and that extend in a single-stranded fashion for at least 12 to 16 nucleotides beyond the complementary strand at the chromosome terminus.[492] DNA ends, in general, are reactive moieties, and telomeres have the potential for being sites of great DNA instability, chromosome-to-chromosome translocation, and occasional loss with cell division. The structure of telomeres, through nucleotide-nucleotide or DNA-protein interactions, seems to stabilize chromosome ends to limit such breakages, fusions, and losses. [493]

### DNA Replication and the End-Replication Problem

There is a particular obstacle to the faithful replication of the ends of chromosomes ( Fig. 4-14A, Fig. 4-14B). DNA polymerases can only add nucleotides onto preexisting nucleotides. Hence, there is a requirement for priming, usually provided by short RNA molecules, in the initiation of DNA synthesis. There is also a directionality to DNA synthesis; one of the two strands of the double helix is synthesized in a 5' to 3' direction, and the other must be synthesized (globally) in a 3' to 5' direction. Because DNA polymerases only add nucleotides in a 5' to 3' direction, replication of the two strands of a double helix must be accomplished in distinct ways. The “leading” strand is replicated continuously in a 5' to 3' direction, providing a directional vector to the replicative fork. The opposing strand is replicated by a succession of priming and 5' to 3' syntheses of short fragments (i.e., Okazaki fragments) that displace the successive primers and are subsequently ligated together (i.e., the lagging strand).

**FIGURE 4-14.** DNA replication, the “end-replication” problem, and the action of telomerase. **A:** Schematic of DNA replication. One strand is synthesized continuously in a 5' to 3' direction (i.e., the leading strand), which moves in the direction of the replicating fork. The other strand is synthesized discontinuously (i.e., Okazaki fragments) in a 5' to 3' direction and is subsequently ligated, (i.e., lagging strand). In both strands, synthesis proceeds from RNA primers. At the ends of the chromosome, the lack of additional template makes RNA priming impossible, and the 5' ends of the newly synthesized strands bear deletions compared with their opposing templates. **B:** Successive rounds of DNA replication as diagrammed in **A** result in gradual shortening of the ends of the chromosomes by one or more telomeric repeat units. **C:** Mechanism of telomerase action. The RNA component of the telomerase complex includes a portion (*white letters on black circles*) that is complementary to the nucleotide repeats on the 3' end of the strand that forms the end of the chromosome. The reverse transcriptase activity of the protein component of telomerase adds deoxyribonucleotides (DNA; *black letters on white circles*) to the 3' end of the strand using the telomerase RNA as a template. The 3' end is elongated by additional telomeric repeat units, with the number of additional repeats determined by the number of times the telomerase complex translocates along the end of the chromosome. The opposing strand is then synthesized in a 5' to 3' direction ( *black letters on white diamonds*) by conventional DNA polymerases. The newly synthesized 5' ending strand does not shorten with each round of replication. Compare this result with the result in **A** and **B**.

The 5' ends of both newly synthesized strands cannot be completely replicated without an additional mechanism being invoked ( Fig. 4-14A). The RNA that primes from the extreme 5' end of the strand cannot be displaced by DNA because there is, by definition, no template to allow the DNA polymerase to accomplish that final displacement. Over time, with each successive cycle of replication, the chromosome is shortened by failure to complete replication of the 5' ends of the double helix (Fig. 4-14B).

### Telomere Shortening and Aging

Models based on the mechanism of DNA replication propose that at some point after a certain number of cell divisions a critical shortening of the ends of chromosomes is reached, chromosomal instability ensues, and the cells die. Telomere shortening is proposed as a “cellular clock” and a mediator of senescence.[494,495 and 496] Support for this model, although mostly correlative, is compelling. For example, when fibroblast cultures were established from individuals of widely different ages, the initial telomere length was found to be proportional to the number of generations that the individual cultures could sustain. Moreover, in cells derived from patients with progeria, a syndrome of premature aging, it was found that telomere length was significantly shorter than that in cells from age-matched controls and that the “progeric” cells had less proliferative capacity. [497] When fibroblasts are serially passed in culture, their telomeres become progressively shorter.[498,499]

The production of sperm and ova must be resistant to the end-replication problem and the successive telomere shortening that accompanies cell division. Otherwise, a species would be incapable of perpetuating itself from generation to generation. By starting off with critically shortened chromosome ends, the next generation would be incapable of maintaining the requisite number of cell divisions for growth and development. Germ cells maintain the size of their telomeres through the action of the enzyme telomerase.[500] It is the absence of telomerase activity in most somatic tissues that initiates the clock mechanism described in the previous section.

### Telomerase Activity

An enzyme activity capable of adding telomeric sequences onto single-stranded oligonucleotides already containing telomeric repeats was first demonstrated in cellular extracts derived from the ciliate *Tetrahymena*, an organism with a large number of chromosomes.[501] Subsequently, the terminal transferase activity was shown to require the presence of a particular RNA molecule in the extract [502]; the RNA sequence included a segment that was complementary to approximately 1.5 telomeric DNA repeat sequences.[503] Analyses of telomerase activity in many different organisms, including humans, have demonstrated the essential consistency of these initial findings. Telomerase is a ribonucleoprotein complex in which the RNA component is used for the recognition and as a template for the extension of telomeric repeats. The complex has terminal transferase and reverse transcriptase activities. [500] After additional telomeric repeats have been added to the strand whose 3' end forms the end of the telomere, the opposing strand is thought to be synthesized by the standard 5' to 3' DNA polymerase activity described in the previous section ( Fig. 4-14C). Additional telomere-recognizing DNA-binding proteins probably place a rough upper limit on the number of additional repeats that telomerase can add. [504]

### Telomerase Reactivation, Immortality, and Cancer

The scenario presented in the section Telomere Shortening and Aging for senescence in cell culture after the loss of telomeric repeats is true without exception. When nonmalignant cells are placed in culture, they do divide for a finite time, after which they die. Certain types of cells, however, more often cells of certain species or strains, may survive this growth crisis and emerge as immortal cell lines. Shortening of telomeres has been observed in cultured cells before their growth crisis; however, the cells that emerge from crisis and are capable of continued growth show a stabilization of their telomere length and a reactivation of telomerase activity.[505] When more than 100 immortal cell lines were assayed, most demonstrated telomerase activity. [506,507 and 508] Equally striking was the finding of telomerase activity in more than 84% of frank malignancies, comprising more than a dozen distinct tumor types. [500, 506,507 and 508]

In the presence of p53 heterozygosity, a new tumor spectrum was observed in mice deficient for the essential RNA subunit of telomerase. When these mice were bred for several generations they experienced the expected shortening of their telomeres, but in addition began to develop the kind of epithelial cancers frequently observed in human populations but occurring only rarely in mice. The tumors were notable for cytogenetic evidence of a preceding period of cataclysmic genomic instability associated with telomere-telomere fusion, breakage-fusion-bridge cycles, translocation, duplication, and deletion—the hallmarks of many human epithelial tumors.[509] A testable model has arisen in which this mutation causing cataclysm is resolved by the reactivation of telomerase stabilizing the reconfigured genome and granting the now transformed cell immortality.[510]

In some cases, levels of telomerase activity may correlate with tumor virulence and prognosis. For example, of 100 neuroblastoma cases analyzed, 94 had detectable telomerase activity.[511] The higher the activity, the more additional genetic changes were apparent and the poorer the prognosis. Three of the tumors that lacked telomerase activity were classified as stage IVS, a form characterized by spontaneous regression.

### Telomeres and Telomerase: Implications for Cancer Therapy

The preceding discussion suggests a possible avenue to overcoming the cancerous state. If, for example, the action of the telomerase RNA-protein complex could be inhibited, there might be a critical shortening of telomeres within the malignant cell, leading to chromosomal instability and death. Research directed toward this and related possibilities has been undertaken in academic and commercial laboratories; however, it is too early to know whether this strategy will be successful for any, all, or no malignant diseases.

The available data indicate several obstacles to this strategy. First, not all malignancies may be immortal, and some cancers may be lethal even with a limited lifespan. Second, some tumors may have telomeres of such length that inhibition of telomerase, although effective, would not result in lethal instability within a therapeutic time frame. Third, there may be telomerase-independent ways of elongating the ends of chromosomes.[512] Nonetheless, if blocking, repressing, or inhibiting tumor-reactivated telomerase works for any cancer, a major and insightful therapeutic breakthrough will have been achieved.

## SUMMARY

Childhood cancer is ultimately a disorder of genes, whose identity and function have preoccupied cell biologists for more than 20 years. The genetic portrait of the diverse group of neoplasms we call *pediatric tumors* is being rapidly filled in. We know that aberrant activation of transcription factor or tyrosine kinase genes by translocation-mediated fusions with normally unrelated partners or by rearrangement to sites near immunoglobulin or TCR genes can lead to acute leukemia, lymphoma, and sarcoma.

It is intriguing that most of such genes have close homologues in the genes controlling *Drosophila* morphogenesis, underscoring their faithful conservation in nature and their relevance to programs of early cell differentiation. The inappropriate expression of specific oncogenes in hematopoietic or mesenchymal progenitor cells could be expected to block normal programs of differentiation.

The tumor suppressor genes, best exemplified by *p53* and *RB*, can give rise to pediatric tumors through an entirely different mechanism. Rather than inappropriate activation (i.e., gain of function), these antioncogenes must be deleted or otherwise inactivated (i.e., loss of function) before their carcinogenic effects become apparent. Every human cancer contains mutations of one or more genes from the oncogene and antioncogene categories, implying a multistep process leading to full-blown malignancy.

Newer classes of cancer genes include defects in nucleotide mismatch recognition and repair, which can lead to hypermutability within cells, a condition ideal for productive mutation of growth-affecting genes and mutations resulting in reactivation of the telomerase gene, whose activity can restore telomere length to chromosomes and thereby confer immortality to cells.

The discovery of key molecular events in the pathogenesis of childhood tumors has not translated into major advances in therapy, but evidence from many fronts, including the treatment of APML with all-*trans*-retinoic acid, suggests that cancer will eventually yield to molecular intervention, assuming that we can find and fully characterize the proper targets.

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# 5

# BIOLOGY OF CHILDHOOD CANCER

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MICHAEL B. KASTAN



## INTRODUCTION

Within the processes of normal cellular development and tissue formation and renewal, cells evolve to perform highly specialized functions. These processes are tightly regulated in response to the physiologic needs of the host. The idealized cell ( Fig. 5-1) has several developmental decisions: continued progression through the cell cycle, differentiation to a specialized cell type, or programmed cell death. The probability that a cell embarks on these paths is influenced by its genotype and its environment. Cells have evolved complex signal transduction pathways that enable them to sense and respond to neighboring cells and their extracellular milieu. These signaling paths influence survival/death, growth/growth arrest, differentiation/undifferentiated, motile/non-motile, and angiogenic/anti-angiogenic decisions and, ultimately, cell fate. Components of these pathways include membrane-bound protein receptors, cytoplasmic/nuclear receptors, phospholipid signaling systems, and ion channels and are highly conserved, being found in yeast, flies, worms, and mammals.

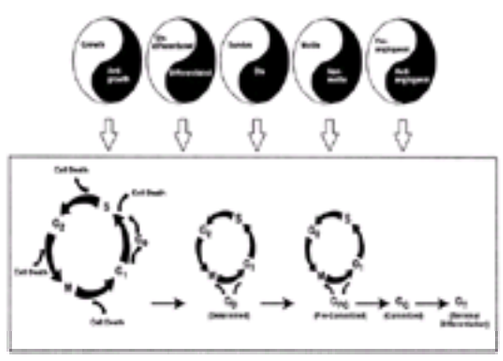

**FIGURE 5-1.** Model of the developmental decisions (proliferation, differentiation, and death) a cell encounters during ontogeny and the potential external influences that shape decisions (growth versus anti-growth; survival versus death; differentiate versus undifferentiate; pro-angiogenic versus anti-angiogenic; motile versus non-motile). Functionally defined stages of cell cycle are S, DNA synthesis; M, mitosis; $G_1$, gap between M and S; and $G_2$, gap between S and M. $G_0$ is the temporary withdrawal from the cell type.

Carcinogenic events can occur at any time during ontogeny or tissue renewal, leading to a tumor composed of cells with distinct developmental characteristics and potentials.[1] *In vitro* carcinogenesis studies in animals indicate that tumorigenesis is a multistep process, functionally defined by initiation, promotion, and progression. [2] Initiation is the alteration of a cell resulting in a heritable change that may affect its fate. Although initiated cells may develop normally, initiated cells may also have alterations in genes that lead to a conditionally lethal state and the induction of cell death or apoptosis. If an initiated cell with a conditionally lethal genetic change acquires a subsequent alteration(s) that suspends or bypasses the lethal state, then the initiated cell may develop into a tumor. Genomic instability, chemicals, growth factors, and hormones produced by the cell or in its environment that alone are not carcinogenic may promote tumorigenesis by suspending a fundamentally lethal condition in an initiated cell. Tumor progression is marked by the ability of a tumor cell to adapt or influence its microenvironment principally by stimulating a vascular supply. The multistep process of tumorigenesis is the result of a series of alterations in genes that function in signal transduction pathways that regulate at least four major parameters of a cell's own fate as well as its interaction with its microenvironment: (a) regulation of cell cycle progression, (b) apoptosis, (c) differentiation, and (d) migration and angiogenesis.[3] Studies indicate tumor cells have different or variable potentials that may include the development of nonmalignant, malignant, and highly malignant or metastatic tumor cells. These potentials reflect the environment in which the tumor cell resides, the acquisition of additional mutations, or a

combination of these factors.[2,3 and 4]

An inherent property of tumor cells is increased genetic instability that may lead to an accumulation of mutations that also influence and alter their biologic properties.[3] In a tumor cell, unlimited proliferation may be favored despite normal cellular and environmental restraints, and characteristics associated with differentiated functions are frequently diminished. This implies that the normal regulatory links between growth control and differentiation have been uncoupled. The ability of chemicals and biologic response modifiers to control cell growth and induce differentiation or cell death in tumor cells suggests that some compounds may bypass altered signal transduction pathways that control these processes.[5,6]

There are two major types of signaling pathways: those whose activities are mediated by membrane-bound receptors and those whose activities are transduced by cytoplasmic or nuclear receptors. The targets of these paths are transcription factors, DNA-binding proteins that regulate the transcription of genes that ultimately control cell function. Members of the membrane-bound signaling pathways include receptors that have an intrinsic kinase activity, such as the epidermal growth factor receptor (EGFR), and receptors that lack an intrinsic kinase activity such as integrins that interact with intracellular kinases (e.g., members of the src family) ( Table 5-1). Another major type of signaling pathway involves cytoplasmic/nuclear receptors, such as hormone, lipid, and vitamin receptors that directly bind DNA sequences in the regions of genes that regulate RNA transcription.

**TABLE 5-1. GROWTH FACTORS AND THEIR RECEPTORS**

The ability of any factor (e.g., polypeptide, vitamin, hormone) to activate a signal transduction pathway is a function of its concentration in the extracellular milieu and the number of receptors expressed by a cell. The intensity of the stimulated signal transduction pathway and its ultimate effects on cell function also depend on the concentration and activation state of the downstream intracellular effectors of the signal transduction pathway. Because the signal transduction pathways of many different receptors use common downstream effectors or intermediates, a cell's response reflects its ability to transmit and integrate a number of different signals that may be incoming at any given time. A cell's response to a factor reflects the convergence of these signals on nuclear transcription factors that specifically activate or repress transcription of genes important in stimulating or inhibiting cell proliferation, cell death, cell differentiation, cell migration, or angiogenesis.

The biochemical paths by which extracellular factors regulate gene transcription and the synthesis of specific proteins that control developmental decisions are complex, as is the role of these proteins in tumorigenesis. They may be directly involved in tumorigenesis, as genetic alterations may occur in proteins within the signal transduction pathway or their target transcription factors, resulting in constitutively active or repressed signal transduction pathways. However, they may also play an indirect role as epigenetic phenomena active in a cell as a part of its normal developmental program that continues to affect the biology of a cell during and after the tumorigenic genotype is established.[7]

A discussion of every growth factor and signal transduction pathway and the intricacies of their interactions is beyond the scope of this chapter. This chapter reviews the current state of knowledge for the major molecular mechanisms regulating cell growth, apoptosis, differentiation, metastasis, and angiogenesis; comments on the involvement of known cancer genes in these processes; and discusses current therapeutic strategies and clinical protocols using drugs and biologics targeted to these pathways.

## GROWTH-REGULATING FACTORS, RECEPTORS, AND SIGNAL TRANSDUCTION PATHWAYS

*Growth factor* is the historical name given to polypeptides, first isolated from the conditioned media of cells grown *in vitro*, that stimulated a cell response such as cell survival or proliferation. One example, transforming growth factor-b (TGF-b), was also called *sarcoma growth factor*, because it stimulated anchorage-independent cell growth, an *in vitro* property of transformed cells.[8] Now, TGF-b is known to inhibit the growth of many cell types.

Many peptides were first identified as hormones [e.g., insulin-like growth factors (IGFs)]. Although the action of these peptides may be endocrine and may occur over great distances, their activities may also be paracrine or local, influencing neighboring cells. Cells may produce factors that are autocrine or autostimulatory. If these factors are secreted, they may be involved in a public autocrine loop, or if the factors are stimulatory even if not secreted, they may be considered as intracrine or stimulating a private autocrine loop. Juxtacrine factors are not secreted but are expressed on the cell surface and influence neighboring cells. Table 5-2 provides a glossary of terms.

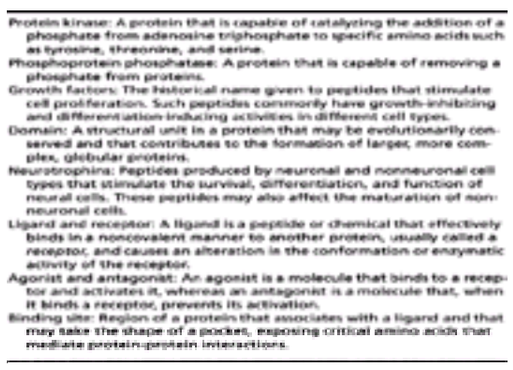

**TABLE 5-2. GLOSSARY**

## TRANSMEMBRANE RECEPTOR PROTEIN KINASES AND THEIR SIGNAL TRANSDUCTION PATHWAYS STRUCTURE

Protein tyrosine (Tyr) kinase receptors consist of a polypeptide chain that may be functionally and structurally separated into distinct domains: the extracellular domain, a transmembrane domain, the juxtamembrane region, the Tyr kinase domain, and the carboxyl-terminal domain (Fig. 5-2).

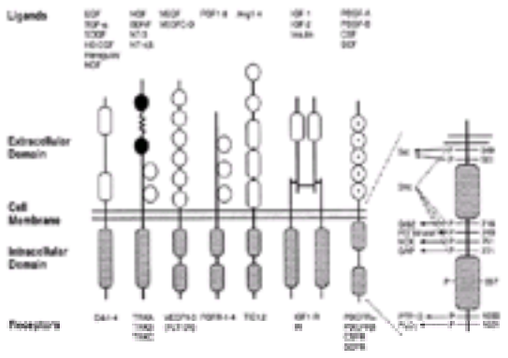


**FIGURE 5-2.** Schematic representation of receptor protein tyrosine kinases. Receptor protein tyrosine kinases contain an extracellular domain that binds ligand, a transmembrane domain that spans the cell membrane (*stippled horizontal line*), and an intracellular domain. The open horizontal bars represent cysteine-rich regions, the open circles to the right of the line are Ig-like loops, and open circles with a dot in the middle are leucine-rich areas. The closed circles separated by a jagged line in the Trk receptor family represent cysteine-rich regions separated by fibronectin type III repeats. The tyrosine kinase domain ( *striped rectangles*) expands to the left of the platelet-derived growth factor (PDGF) receptor to illustrate the known tyrosine amino acids ( *numbered*) that are phosphorylated (P) and known intracellular proteins or protein motifs that bind to specific phosphorylated tyrosines after ligand binding and receptor dimerization. Ang 1-4, angiogenesis stimulating factors; BDNF, brain-derived neurotrophic factor; CSF, colony-stimulating factor; EGF, epidermal growth factor; ErbB1-4, epidermal growth factor receptors; FGF, fibroblast growth factor; GAP, Ras-GTPase-activating protein; HB-EGF, heparin-binding EGF-like growth factor; IGF, insulin-like growth factor; IR, insulin receptor; NDF, neu differentiation factor; NGF, nerve growth factor; NT, neurotrophin; R, receptor; SCF, stem cell factor; SDGF, schwannoma-derived growth factor; TGF, transforming growth factor; Tie 1,2, receptors for Ang; TRK, tropomyosin receptor kinase; VEGF, vascular epithelial growth factor.

The extracellular domain binds ligand. Ligands may be monomers that may bind two receptors simultaneously [e.g., epidermal growth factor (EGF)], disulfide-linked dimers (e.g., platelet-derived growth factor), or noncovalently linked dimers [e.g., stem cell factor (SCF)]. In addition to binding ligand, the extracellular domain is characterized by several motifs, such as cysteine-rich domains, immunoglobulin (Ig)-like loops, leucine-rich motifs, fibronectin-like repeats, and EGF-like repeats, that may be involved in ligand binding, stabilization of the receptor dimer, or non-ligand–mediated receptor-receptor interactions. Ligand-receptor interaction may cause an allosteric change in a receptor or dimerization, or both, or oligomerization of receptors that lead to activation. The transmembrane region anchors the receptor in the lipid bilayer of the cell membrane, and the juxtamembrane region separates the transmembrane domain from the cytoplasmic domains. Upon ligand binding and receptor dimerization, Tyr residues in this region are autophosphorylated and mediate interactions with cytoplasmic binding proteins. [6]

The amino acid sequence of the Tyr kinase domain is the most highly evolutionary conserved domain among different receptors, and single amino acid alterations in the adenosine triphosphate–binding site can lead to a loss of function. The catalytic activity of the Tyr kinase domain involves the ability of the protein to phosphorylate neighboring Tyr residues. Phosphorylation of key residues in the kinase domain can increase its catalytic activity. Protein phosphorylation at Tyr, serine, or threonine amino acid residues by kinases and dephosphorylation by phosphatases represents a major component of the “signal” in signaling pathways. Phosphorylation plays a critical role in biologic processes because it can rapidly alter protein activity or alter protein-protein interactions. [9] Forms of receptors lacking the Tyr kinase domain have been identified in an increasing number of receptors, including receptors for neurotrophins and fibroblast growth factor. Although their precise role is not clear, truncated receptors may have unique signaling pathways, facilitate ligand binding, or act as dominant negative proteins that, by binding a ligand, sequester it from kinase-active receptors. The carboxyl-terminal domain contains several Tyr residues that are phosphorylated by the kinase domain. The ability to make site-directed mutants of various residues in this regions has defined the importance of these residues and delineated their intracellular intermediaries in signaling pathways.

## SIGNAL TRANSDUCTION PATHWAYS

Ligands, which may exist as monomers or dimers or remain cell bound, may interact with receptors and activate several intracellular signal transduction pathways: a mitogen-activated protein kinase (MAPK) pathway,[10] a protein kinase C (PKC) pathway,[11] or the phospholipase C-g (PLC-g) signal transduction pathway. [12] The members of these pathways, their mechanisms of activation, and their interactions are under intense investigation, and, because of their complexity, the models presented here are schematic (Fig. 5-3).

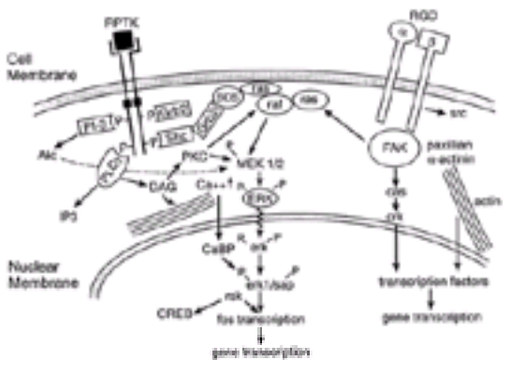


**FIGURE 5-3.** Schematic diagram of common signal transduction pathways used by growth factor receptors and cell adhesion molecules. Growth factors ( *squares*) or RGD-containing proteins (RGD) interact with membrane-bound receptors, facilitating receptor dimerization or oligomerization that initiates the signal transduction cascade. In the case of receptor protein tyrosine kinases (RPTKs), activation involves enhanced activity or intrinsic receptor kinase, and with cell adhesion molecules that lack an intrinsic kinase, activation leads to interaction with intracellular protein kinases that propagate the signal. Solid arrows represent predominant signal transduction pathways activated, and dotted lines indicate ancillary activation pathways. Ca, calcium; CaBP, Ca-binding protein; DAG, diacylglycerol; FAK, focal adhesion kinase; IP3, inositol-3-phosphate; PI-3, phosphotidylinositol-3; PKC, protein kinase C; PLCg, phospholipase C-g.

Ligand-receptor interaction leads to homoreceptor or heteroreceptor dimerization, followed by the reciprocal autophosphorylation of Tyr residues in the cytoplasmic region of one receptor by the kinase domain of the other receptor. [13] Tyr residues within the catalytic domain are phosphorylated (with the exception of the EGFR), and this may enhance receptor kinase activity. Phosphorylation of other Tyrs in the juxtamembrane region and the carboxyl terminus creates docking sites for downstream signal transduction proteins.

The binding of the receptor to downstream partners in the signal transduction pathway and the binding of these intermediaries to each other are mediated by specialized domains within the proteins. [14,15] SH2 and SH3 were originally identified as src homology 2 (SH2) and 3 (SH3) domains. SH2 is a 100 amino acid domain whose tertiary structure forms a binding pocket for a phosphorylated Tyr (PTyr) and the immediate surrounding amino acids found on a receptor. These domains recognize short linear stretches of four to ten amino acids usually surrounding a phosphorylated Ser/Thr or Tyr. SH2, SH3, PTB, WW, FHA, SAM, LiM, PX, EH EVH1, and PDZ are examples of protein-protein interaction domains. [15] These domains mediate alterations in protein conformation that change the catalytic activity of interacting proteins or alter subcellular protein location, concentrating proteins to the cell membrane or cytoskeleton. Differences in the binding affinities of these protein domains to receptors can influence signal transduction. If multiple receptors are activated in a cell at the same time, there may be competition for these proteins, leading to signal “squelching” or signal amplification or to activation of additional pathways, a process called *crosstalk*.

Receptors can directly activate signaling pathways by binding to proteins that have intrinsic kinase activities, such as src or phosphatidylinositol-3 kinase (PI-3 kinase), or that activate second messengers, such as PLC-g. The SH2 domain in PLC-g binds a specific PTy in the receptor, leading to the formation of inositol-1,4,5-triphosphate and diacylglycerol. This leads to a slow release of $Ca^{2+}$, which can bind and alter the activity of a number of $Ca^{2+}$-binding proteins, such as calmodulin and PKC, both ubiquitous modulators of protein kinases and other enzymes. [16,17] Diacylglycerol is also potent activator of PKC. Numerous compounds

identified as tumor promoters are strong activators of PKC.[16] Proteins can also directly interact with lipids via the “pleckstrin” homology domain, which recognizes specific phospholipids and enables membrane associations dependent on lipid second messengers.

The identification of adapter proteins, such as Grb, IRS-1, Shc, Crk, and Nck, that bind specific receptor PTy coupled Tyr kinase signals to the MAPK and ras signaling pathways.[18] Although adapter proteins lack intrinsic kinase activity, they function in many receptor signaling pathways by binding PI-3 kinase and PLC-g. The adapter protein Grb2 binds to the guanine nucleotide exchange factor, Sos, which activates ras-guanine triphosphatase (GTPase) activity. Activated ras stimulates the MAPK pathway by recruiting Raf to the plasma membrane, where it is activated by another, unknown signal from the Tyr kinase. The MAPK pathway may be viewed as a model for a growing number of signal transduction pathways that have as their core three serine-threonine kinases that are sequentially activated; a serine-threonine protein kinase (MAPKKK) that phosphorylates and activates a dual specificity serine-threonine kinase (MAPKK), which phosphorylates and activates another serine-threonine kinase (MAPK).[10] In Figure 5-3, the Raf functions as the MAPKKK, and its activation initiates the signal cascade, phosphorylating and activating Mek, the MAPKK, which is a dual-specificity serine-threonine kinase that phosphorylates and activates extracellular signal-regulated kinases (ERKs), the MAPK.

Activation of transcription factors by extracellular signals can occur at the cell membrane, in the cytoplasm, or in the nucleus, but it is usually accompanied by a nuclear translocation step.[19] Primary substrates for the MAPK (i.e., ERK) are transcription factors such as Elk-1 or Sap-1. Elk and Sap are major regulators of the promoter region of the transcription factor Fos. Transcription factors act as homodimers or heterodimers and frequently as multiprotein complexes that coordinately regulate gene transcription. A number of transcription factors were originally identified as viral oncogenes (e.g., jun, fos, myc, myb, rel, and ets), and synthesis of their normal counterparts is part of the immediate or early response of cells to growth factor stimulation. Ultimately, these transcription factors participate in the regulation of genes controlling progression through the cell cycle, DNA replication, metastasis, angiogenesis, cell differentiation, and cell death.

## EPIDERMAL GROWTH FACTOR FAMILY

### Epidermal Growth Factor and Its Receptor

EGF was initially identified in extracts of murine submaxillary glands as a factor that promoted premature eyelid separation and tooth eruption by enhancing epidermal growth and differentiation.[20,21,22] and [23] Extensive studies documented its ability to stimulate epidermal cell proliferation in cell and organ cultures *in vitro*.[21] The human homologue was first characterized as a factor, urogastrone, that inhibited gastric secretion and stimulated the differentiation of rat and human intestine. Although EGF and urogastrone had seemingly different properties, amino acid analysis revealed that the factors were identical. [24] Mature EGF is composed of 53 amino acids and three internal disulfide bonds. It is derived by proteolytic cleavage from a precursor molecule of 120 amino acids that consists of eight EGF repeats and a hydrophobic carboxyl terminus. The EGF precursor molecule can be found as a glycosylated membrane–bound protein and may function as a juxtacrine growth factor.

The EGF family includes TGF-a, which is normally found in epithelial cells; amphiregulin, which is found in human placenta, ovarian, and breast tissue; neuregulin 1-3, which is detected in mammary tissue and Schwann cells; heparin-binding EGF-like growth factor (HB-EGF); and betacellulin, which is most highly expressed in lung, uterus, and kidney. EGF-induced crosslinking identified a 170-kd receptor that contains two extracellular cysteine-rich domains ( Fig. 5-3). Isolation of the gene encoding the EGF receptor (ErbB-1) and its use in low-stringency screening led to the identification of additional members of this family: ErbB-2, ErbB-3, and ErbB-4. EGF and TGF-a bind primarily to ErbB-1, causing receptor dimerization. Neuregulin 1-3 primarily binds to heterodimers of ErbB-3 and ErbB-4. Betacellulin and HB-EGF primarily bind to ErbB-1 and ErbB-4. ErbB-2 may be considered an orphan receptor as it has no known high-affinity ligand, although it does form heterodimers with ErbB-1, ErbB-3, and ErbB-4. Overexpression of ErbB-2 may lead to receptor dimerization of ErbB-2 in the absence of ligand and constitutive activation of the receptor. The structure of ErbB-3 lacks several highly conserved amino acid residues in its kinase domain, and it is unclear whether the receptor contains an intrinsic kinase activity. ErbB-3 may serve as a substrate for the ErbB-2 kinase activity in the heterodimer and provide docking sites for downstream SH2 domain-containing signal transduction intermediates. Some experiments indicate that a kinase-negative mutant of ErbB-1 is capable of activating the MAP kinase pathway, but is not able to induce phosphorylation of GTPase-activating proteins. [25] Studies in the fruit fly indicate that the principal EGF signaling path is EGFR>Grb2>SOS>RAS>RAF>MEK>MAPK > nucleus.[25] Relevant properties of some members of this family are summarized in Table 5-1.

### Role of Epidermal Growth Factor Family in Cancer

High levels of EGF, TGF-a, and their receptors have been reported in glioblastoma and head, neck, breast, and bladder cancer tumor specimens, and an autocrine mechanism of growth has been observed in several of these tumor cell models.[26] High levels of HER2/neu (ErbB-2) with or without gene amplification are found in ductal carcinoma of the breast squamous cell carcinomas of the lung and most adenocarcinomas of the lung. [27] In breast and lung cancer, high-level expression is associated with shorter overall survival, and in breast cancer, it also correlates with a shorter relapse-free survival and a failure to respond to hormonal therapy. [28] In neuroblastoma (NB), the median survival of patients whose tumors stained with HER2/neu antibodies was 12 months, whereas those whose tumors were negative survived 138 months.[29]

As single agents, several monoclonal antibodies against EGFR produce a tumorstatic response in mice xenograft models, whereas in combination with chemotherapeutics and cytokines such as tumor necrosis factor-a (TNF-a), the response is tumoricidal. Inhibition of the EGF signal transduction path decreases cell proliferation, and, in some cell types, this is accompanied by the induction of apoptosis. Furthermore, some studies indicate that anti-EGFR antibodies decrease pro-angiogenic factors in tumor cells[30] and inhibit metastases.[31] Selected radiolabeled anti-EGFR and anti-HER2/neu antibodies (e.g., MAb4D5) are under clinical evaluation as imaging agents and for their antitumor activity. [30] Trastuzumab (Herceptin) is a humanized monoclonal antibody against the EGFR that has been in clinical trials primarily in patients with ErbB-2 over-expressing breast cancer. A 24% partial and complete remission was seen in patients without prior chemotherapy compared to a 11% to 14% response in patients receiving chemotherapy. Patients receiving Trastuzumab and chemotherapy have a 24% reduction in risk of death as compared to those receiving standard chemotherapy.[32]

## FIBROBLAST GROWTH FACTOR FAMILY

### Fibroblast Growth Factors and Their Receptors

Basic fibroblast growth factor (bFGF) was originally identified in extracts of pituitary cells on the basis of its ability to stimulate fibroblast and chondrocyte cell proliferation.[33] Properties of FGFs are summarized in Table 5-1. The ability of FGFs to bind heparin facilitated their purification and identified other members that were also known as heparin-binding growth factors.[34,35] Because monomeric FGF interacts with the receptor, heparin induces oligomerization of FGF, leading to FGF receptor (FGFR) dimerization and activation.[36]

There are nine members in the FGF family: acidic FGF (aFGF or FGF-1), bFGF (or FGF-2), int-2 (FGF-3), hst/KS3 (FGF-4), FGF-5, FGF-6, keratinocyte growth factor (KGF or FGF-7), androgen-induced growth factor (AIFG or FGF-8), and glia-derived growth factor (GAF or FGF-9). FGFs stimulate the growth of cells of mesenchymal, neuroectodermal, and epidermal origin, and many stimulate angiogenesis. bFGF and aFGF, derived from an acid extract of bovine brain, are single polypeptide chains of 17 kd that share approximately 55% amino acid sequence homology. These peptides bind glycosaminoglycans and are highly concentrated in extracellular matrix. FGF-2 was originally identified as int-2, a common integration site for mouse mammary tumor virus. Normal expression of int-2 is limited to embryonic tissues, but in int-2 transgenic mice, females have mammary gland hyperplasia and males have benign prostate epithelial hyperplasia. [37] FGF-4 was first identified as an oncogene from a human stomach tumor (hst) and a Kaposi's sarcoma (KS3).[38] FGF-5 was identified as a transforming gene caused by the insertion of a retroviral promoter upstream of its own promoter and leading to a constitutive gene transcription. [39] FGF-7 or KGF is unique among the members of this family, because it stimulates keratinocyte cell proliferation but not fibroblast or endothelial cell proliferation. Analysis of the FGF-7 gene placed it in the FGF family; it had 38% amino acid homology with aFGF and bFGF. It is highly expressed by stromal cells and may be an important regulator of epithelial cell proliferation. [40]

There are four major FGF receptors (FGFR-1 through FGFR-4) and a number of receptor isoforms that are generated by differential messenger RNA (mRNA) splicing (Fig. 5-3).[41] Cell- and tissue-specific RNA processing in the regions encoding the three extracellular Ig-like disulfide loops leads to variant FGFRs with differences in receptor binding activity and specificity. FGF stimulation of an FGFR carrying a mutation in Tyr766 fails to bind PLC-g and stimulate hydrolysis of phosphatidylinositol or increase $Ca^{2+}$ mobilization; however, disruption of this signaling pathway does not prevent FGF-induced mitogenesis. [42]

### Role of Fibroblast Growth Factor Family in Cancer

The products of hst/KS and fgf-5 members of the FGF family were identified by their ability to transform NIH-3T3 cells, and int-2 is implicated in mouse mammary tumor virus–induced murine mammary tumors. Overexpression of bFGF is also capable of transforming cells. When an Ig signal sequence was linked to the bFGF, the transforming ability was dramatically increased, suggesting that altering bFGF to a secreted form may be transforming in itself. The products of hst/KS, fgf-5, and int-2 all contain signal sequences and are more likely to be secreted. [42,43 and 44]

The finding of FGFs in normal brain tissue led to analysis of their expression in tumors. FGFs are expressed in human gliomas, astrocytomas, and glioblastoma multiforma.[45,46 and 47] Increased expression of an alternatively spliced variant of FGFR-1 and decreased expression of FGFR-2 is detected in more malignant astrocytomas compared with lower-grade tumors or normal cells.[48] Antisense oligonucleotides to FGF inhibit the growth of transformed astrocytes or glioblastoma cells, indicating an FGF-mediated autocrine growth mechanism and identifying a potential therapeutic target. [49,50]

FGFs may contribute to oncogenesis by their ability to stimulate tumor angiogenesis. Tumor angiogenesis appears to be a key feature in the development of solid tumors. Both aFGF and bFGF are potent stimulators of angiogenesis in various *in vivo* assays.[51,52] *In vitro*, FGFs are capable of acting as mitogens and chemotactic factors for endothelial cells and stimulating protease secretion from these cells, which may also be important in tumor angiogenesis and tumor metastasis. [53] The inhibition of FGF-induced tumor angiogenesis may provide a new target for anticancer therapy.

## INSULIN-LIKE GROWTH FACTOR FAMILY

### Insulin-Like Growth Factors and Their Receptors

IGFs were first identified as serum factors, named *somatomedins*, that interacted with growth hormone to stimulate skeletal tissues. [21,22 and 23,51,54] Somatomedin C is now called *IGF-1*, and multiplication stimulating factor is referred to as *IGF-2*. IGF-1 and IGF-2 are 7.5-kd, single-polypeptide chains, sharing 48% and 50% amino acid homology with insulin, respectively. Both IGFs share 70% amino acid homology and are synthesized as prepropeptides whose carboxyl-terminal amino acids are cleaved to yield a 70 amino acid acidic basic IGF-1 protein and a 67 amino acid basic IGF-2 peptide. [55]

IGF-binding proteins (IGFBPs) are a family of proteins that bind to IGFs with high affinity and specificity and are involved in the vascular transport of IGF. IGFBPs modulate IGF interaction with receptors and regulate IGF growth-promoting activity. At least six distinct proteins (IGFBP-1 through IGFBP-6) have been identified and cloned.[56] IGFBP-4 enhances IGF proteolysis, and IGFBP-5 prevents degradation. IGF-1 and IGF-2 predominantly circulate as a complex with IGFBP-3 and an acid-labile glycoprotein. IGFBP-3 may inhibit or stimulate growth *in vivo* and *in vitro*. However, proteolytic cleavage results in IGFBP fragments with decreased binding affinity for IGFs. This serves to regulate free IGF and IGFBP levels in serum and tissues. IGFBPs may also affect cell function independent of IGFs and IGF receptors.

Three membrane-bound receptors bind IGFs: the insulin receptor (IR), the IGF-1 receptor (IGF-1R), and the IGF-2 receptor (IGF-2R). [57] The IR and IGF-1R are structurally related, membrane-bound Tyr kinase receptors. They have a unique heterotetrameric structure composed of two disulfide bounded alpha chains, containing the ligand-binding domain, that are linked by two disulfide bonds to two transmembrane beta chains that contain the intracellular kinase domain ( Fig. 5-3). The IGF-1R binds IGF-1 with the highest affinity, followed by IGF-2 and insulin. [58] The IGF-2R is a transmembrane protein with a short cytoplasmic region and is homologous to the mannose-6-phosphate receptor. The IGF-2R binds both IGFs, but not insulin. Evidence links IGF-2R to a G-coupled receptor signaling system. [59]

Unlike other Tyr kinase receptors, the IGF-1R exists in the membrane as a heterodimer. Ligand binding is thought to cause a conformational change in the dimeric receptor that initiates signal transduction. [60] Key to IGF-1R signal transduction is the phosphorylation of the IR substrate-1 (IRS-1), which contains more than 50 potential Tyr, threonine, and serine phosphorylation sites that, when phosphorylated, mediate interactions with a number of signaling intermediaries. Phosphorylated IRS-1 binds and activates PI-3 kinase and binds Grb2, linking IGF-1R to the ras-raf signaling pathways. In quiescent fibroblasts, IGFs induce immediate early gene transcription (e.g., fos and myc) in early $G_0/G_1$ and make cells competent to progress through the cell cycle after stimulation with platelet-derived growth factor (PDGF) in late $G_1$.[61] Antibodies to ras but not to other G proteins alter IGF function in late $G_1$ but not in early $G_0/G_1$, indicating that the ras pathway mediates a cell's competence to enter S phase.[62]

IGFs are mitogenic in a variety of physiologic conditions, including compensatory organ hypertrophy, nerve regeneration, and wound repair. [63,64,65 and 66] IGFs act synergistically with other growth factors to stimulate DNA synthesis. [64,67] IGFs induce differentiation in myoblasts, chondroblasts, osteoblasts, and neuroblasts and can rescue c-*myc* overexpressing cells from programmed cell death under certain conditions. [68,69 and 70] IGFs also stimulate cell motility.[71,72] IGF-1 appears to mediate the action of growth hormone on skeletal cartilage formation. Because high levels of IGF-2 have been found in cerebrospinal fluid, there may be a role for it in the central nervous system.[73] IGF-1R knockout mice ($IGFR^{-/-}$) are only 30% the size of their normal litter mates, indicating a role for signals mediated by IGF-1R in embryonal growth.[74] Fibroblasts derived from these $IGFR^{-/-}$ mice grow slower in culture, with all phases of the cell cycle protracted. $IGFR^{-/-}$ fibroblasts are unable to grow in serum-free medium, even if supplemented with other growth factors; however, re-introduction of IGFR into these cells by gene transfections renders them sensitive to growth factor stimulation. The IGFR is necessary for cells to be competent to proliferate. [75]

### Insulin-Like Growth Factors and Cancer

Many human tumors have increased IGF-1 ligand or receptor expression, suggesting an autocrine or paracrine role in their growth or survival. These tumors include breast carcinomas, colon carcinomas, hepatocellular carcinomas, lung carcinomas, liposarcomas, and pancreatic carcinomas. [76,77,78,79,80 and 81] Overexpression of IGF-2 mRNA has also been found in breast and colon carcinomas, leiomyosarcoma, pheochromocytoma, hepatocellular carcinoma, Wilms' tumor, NB, and rhabdomyosarcoma.[82,83,84,85,86 and 87] Prostate-specific antigen (PSA) is an IGFBP-3 protease.[88] IGFBP-3 fails to inhibit IGF-1–stimulated growth of prostatic epithelial cells if cultured in the presence of PSA.[89]

IGF-2 may play a role in several pediatric tumors, including Wilms' tumor, rhabdomyosarcoma, and NB. Wilms' tumors express high levels of IGF-2 mRNA, and a blocking monoclonal antibody to the type I receptor (aIR-3) inhibits the growth of tumor heterografts in nude mice. [90] The Wilms' tumor suppressor gene, WT1, is a DNA-binding, serine- and proline-rich, $Zn^{2+}$-binding transcription factor that recognizes several DNA sequences, one of which is a specific binding site for members of the early growth response family of transcriptional activators. [91] Consistent with its role as a suppressor factor, WT1 represses the activity of the IGF-2 promoter and the IGF-1R promoter.[91,92] WT1 mutation, deletion, or underexpression in Wilms' tumor may result in increased expression of the IGF-2/IGF-1R signal transduction pathway. Rhabdomyosarcoma cell lines secrete IGF-2 and grow in serum-free media. The growth of rhabdomyosarcoma cell lines is inhibited by aIR-3. [72] IGF-2 is uniformly expressed by rhabdomyosarcoma tumors, suggesting an autocrine growth loop in most of these tumors.[93] Normally, the IGF-2 gene is silent or imprinted at the maternal allele. In Wilms' tumor and rhabdomyosarcoma, however, evidence indicates that imprinting has been lost, and this may also contribute to the overexpression of IGF-2 in these tumors.[94,95] Although an autocrine role for IGF-2 has been identified in an NB cell line, subsequent studies in tumor tissue rarely detected IGF-2 expression in tumor cells. In most NB tumors, IGF-2 expression was detected in stromal tissues, suggesting a possible paracrine role for this growth factor in NBs growth or survival.[88,89]

Known oncogenes, such as SV40 large T and activated ras, fail to transform fibroblasts from $IGFR^{-/-}$ animals, indicating that the expression of an oncogenic protein alone is not sufficient to transform cells. Transfection of IGFR into $IGFR^{-/-}$ fibroblasts restores the transforming activity of these oncogenes. The necessity of upstream signals, such as IGFs, to induce tumors indicates that IGFs, perhaps by their overexpression, can act as tumor promoters. The concept of IGF-2 acting as a tumor promoter has also been proposed based on studies of transgenic mice that overexpress IGF-2 at puberty. After a long latency period, these mice have a high incidence of hepatocellular carcinoma, sarcomas, and lymphomas, suggesting a role for IGFs as a tumor progression factor. [96]

## PLATELET-DERIVED GROWTH FACTOR FAMILY

The platelet-derived growth factor (PDGF) family is composed of PDGF-A and PDGF-B, vascular endothelial growth factor (VEGF), vascular permeability factor, colony-stimulating factor-1, and SCF. There is limited sequence homology among these peptide factors, and their assignment in this family is based predominantly on the structure of their receptors. Their receptors contain five Ig-like extracellular domains and an insert in their Tyr kinase domain ( Fig. 5-3).

### Platelet-Derived Growth Factor and Its Receptor

PDGF was originally identified as a mitogen derived from platelets that stimulated cells of mesenchymal origin. It consists of two heat-stable polypeptide chains designated A and B that are found as heterodimers or homodimers.[97,98] The PDGF receptors (PDGFRs) are encoded by two distinct genes, alpha and beta, which can also form homodimeric or heterodimeric receptors.[99,100] Ligand specificity is determined by the type of homodimeric or heterodimeric receptor formed; A chains bind only a-PDGFR, and B chains bind a-PDGFR or b-PDGFR. The response to PDGF depends on the isoform of PDGF and on the number of a-PDGFR and b-PDGFR on the responding cell.[101]

The intracellular portion of the b-PDGFR contains at least nine identified autophosphorylation sites that, when phosphorylated, bind distinct SH2-containing proteins (Fig. 5-4). Differences in signal transduction have been observed between aa-PDGFR and bb-PDGFR homodimers that may be due to the differential ability of the cytoplasmic domains to bind intermediary signaling proteins. PDGF-AB induces ab-PDGFR heterodimers and a stronger mitogenic response than detected in homodimeric receptors. This action is due to a unique autophosphorylation site found in the heterodimeric receptor that may interact with additional signaling intermediaries.[102] PDGFR binds the SH2 domain of a protein Tyr phosphatase protein (SH-PTP2), and this may bind Grb2 and mediate activation of ras. Evidence also links the PDGFR to activation of an Akt serine-threonine kinase.[17]

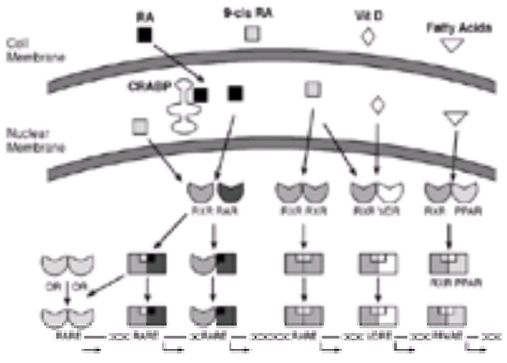


**FIGURE 5-4.** Schematic of cytoplasmic and nuclear receptor regulation of gene transcription. Free hormone or protein-bound hormone (not pictured) transverses the cell membrane, where it interacts with cytoplasmic binding proteins (CRABPs) that may regulate transport or metabolism or nuclear hormone receptors. Retinoic acid receptors (RARs), retinoid X receptors (RXRs), vitamin D receptors (VDRs), thyroid receptors, peroxisome proliferator receptors (PPARs) and orphan receptors (ORs) are depicted by the circular symbols; on dimerization and ligand binding, the receptors are capable of recognizing and complexing with specific response elements (REs) in the promoters of genes. RARE, retinoic acid response element. (Adapted from Pfahl M. Signal transduction by retinoid receptors in skin. Pharmacology 1993;6:8.)

SCF, which is also known as *steel factor*, *Kit ligand*, or *mast cell growth factor*, is a secreted and membrane-bound glycoprotein dimer with a molecular weight of 70,000 to 90,000 that plays a role in hematopoiesis, melanogenesis, and gametogenesis. Its receptor is c-kit, a Tyr kinase. Alterations in SCF, which maps to the murine Sl or steel locus, or c-kit, which maps to W or the white-spotted locus, cause defects in murine coat color. C-kit is the normal homologue of the v-kit oncogene from a feline sarcoma virus, in which the transforming gene contains a deletion in a region that includes the transmembrane domain.[103]

### Platelet-Derived Growth Factor and Cancer

The gene encoding PDGF-B is the human homologue of the simian sarcoma virus oncogene, v-sis.[104,105] This finding was the first clue linking alterations in growth factors to tumor formation. Simian sarcoma virus can infect many cell types but only transforms cells that express PDGFR, suggesting that autocrine stimulation of cell proliferation is associated with transformation. The finding that overexpression of PDGF-B or c-sis also induces cellular transformation [106] led to several studies that identified high levels of PDGF-A chains in a number of human tumors, including osteosarcoma, melanoma, and glioblastoma.[107,108] and [109] In a study of 50 glioblastoma multiforme and anaplastic astrocytoma tumors, 26% of tumors evaluated had amplification of the a-PDGFR or EGFR genes, and an additional 14% had increased protein levels of these genes.[110] A subgroup of chronic myelomonocytic leukemia (CMML) was shown to contain a t(5:12)(q33;p13) involving the fusion of the b-PDGFR Tyr kinase domain to tel, a novel ets-like gene. It remains to be determined what role this chimeric receptor plays in CMML, or if it is involved in the progression of CMML to acute myeloid leukemia (AML).[111] In a subset of ovarian cancers, 10% contain amplification of a downstream effector of PDGF, the Akt serine-threonine kinase.[17]

Autocrine SCF/c-kit signal transduction pathways have been found to regulate the growth of NBs and glioblastomas.[112,113] The c-kit receptor is also highly expressed in some leukemias and may be associated with a myeloid phenotype. In adults with AML, c-kit expression is associated with a poor response to chemotherapy, but in children, expression of c-kit by AML blasts does not predict a poor response to chemotherapy.[114]

## NEUROTROPHINS AND THEIR RECEPTORS

Nerve growth factor (NGF) was first identified as a peptide that stimulated neurite extension in sympathetic ganglion cultures.[115] It is the prototype of the neurotrophin family of peptides that includes brain-derived neurotrophic factor (BDNF), NT-4/5, and NT-3. Neurotrophins encode proteins of 12 to 15 kd and are expressed in a wide variety of neuronal tissues and tissues (e.g., muscles) that require innervation. Neurotrophins stimulate the survival, maturation, and differentiation of discrete but sometimes overlapping populations of neurons and exhibit a developmentally regulated pattern of expression.[116]

The action of neurotrophins is mediated by the trk Tyr kinase receptors. The trk gene was originally isolated as a transforming gene from a human colon carcinoma. It was created by the fusion of the 5' region of the constitutively expressed tropomyosin gene to the Tyr kinase domain of a novel member of the receptor Tyr kinase family; hence, the acronym trk stands for tropomyosin receptor kinase.[117] *In situ* analysis of c-trk indicated that it was highly expressed in neuronal cells, and subsequently trk was identified as the Tyr kinase receptor for NGF. The trk Tyr kinase receptors consist of TrkA, TrkB, and TrkC and alternately spliced variants of TrkB and TrkC that lack the intracellular Tyr kinase domain. Neurotrophins activate Trks through ligand-stimulated receptor Tyr phosphorylation. The p140TrkA binds NGF and, weakly, NT-3. The p145TrkB is activated by BDNF, NT-3, and NT-4/5, and p140TrkC is activated by NT-3. Trk receptors are differentially expressed during development.[118]

Activation of Trk receptors can cause mitogenic signals in fibroblasts [119] or differentiation signals in neural cells.[120] Analysis of signal transduction pathways indicates that the growth-stimulating and differentiating responses induced by activation of Trk receptors involves PI-3 kinase, PLC-g, and ras activation.[121] All neurotrophins also bind to a pan-neurotrophin receptor, NGFR, that is a member of the death receptor family of receptors. Signals mediated by NGFR may be survival or cell death, depending on the cell type and whether Trk receptors are activated.

## TRK RECEPTORS AND CANCER

Oncogenic trk fusion genes are found in high frequency in medullary thyroid cancer but rarely in colon carcinoma, from which it was first derived.[117,122] Relatively high levels of TrkA mRNA and protein expression are detected in NB tumors from patients with a good prognosis, with little expression detected in tumors from patients with a poor prognosis.[123,124,125,126] and [127] Poor-prognosis, advanced-stage tumors primarily express TrkB mRNA and its ligand, BDNF.[128] Differential Trk expression may affect the biology of NB tumor cells with activation of TrkA, leading to growth arrest or apoptosis, whereas activation of TrkB stimulates cell survival, induces neurite extension, and increases cell invasiveness, a characteristic of metastatic cells.[124,129] It is possible that the differences in clinical course of NB patients reflect differences in the basic biology of the cell (i.e., a distinct lineage or maturation state) when tumorigenesis occurred.

Trk immunoreactivity is also detected in medulloblastoma, Ewing's sarcoma, peripheral neuroepithelioma, Wilms' tumor, rhabdomyosarcoma, and glioblastoma. [132,133] and [134] An analysis of medulloblastoma tumors indicates that some patients who had a longer disease-free survival interval expressed relatively high levels of TrkC mRNA and its ligand NT-3.[135] CEP-751, which interferes with the Trk signaling, inhibits the growth of NB and medulloblastomas in a pre-clinical mouse model. [136]

## GLIAL-DERIVED NEUROTROPHIC FACTOR/REARRANGED DURING TRANSFECTION TYROSINE KINASE

RET, which stands for rearranged during transfection, was a laboratory artifact in which lymphoma DNA rearranged during a transfection was found to transform NIH 3T3 cells.[137] Sequence analysis indicated that one of the rearranged genes, RET, was a membrane-bound Tyr kinase receptor. Many properties of RET are similar to Trk; both are highly expressed in neural cells and rearrangements of RET and Trk are frequent in papillary thyroid cancer and lead to kinase active receptors. Transfection of RET and Trk into fibroblasts triggers mitogenesis, but transfection into neuronal cells stimulates differentiation. [139] Studies using mutant RET genes in which the kinase is constitutively active have identified Shc, Grb2, and PLC-g as signaling intermediaries. [138]

Glial-derived neurotrophic factor (GDNF) is a RET ligand. [139] GDNF is a member of the TGF-b family of ligands. GDNF binds to GDNFR-a (GDNFR-a1 to 4), which is anchored in the membrane by glycosyl-phosphatidylinositol and is unable to signal on its own. GDNF activation and binding to RET requires GDNFR-a binding. The dependence of the GDNF/RET signal transduction path on the GDNFR-a accessory receptor is similar to the TGF-b signal transduction pathway except that RET is a Tyr kinase receptor. Other ligands utilizing the RET/GDNFR-a signaling path include neuturin, artemin, and persephin. [140]

Eighteen different mutations in five different codons of the RET gene are associated with the inheritance of multiple endocrine neoplasia type 2A (MEN2A), type 2B (MEN2B), and familial medullary thyroid carcinoma.[141,142] These syndromes are inherited endocrinopathies characterized by medullary thyroid carcinoma, and MEN2A and MEN2B may include pheochromocytoma and ganglioneuromas. Mutations in MEN2A occur in the extracellular binding domain, and a large number of mutations in MEN2B occur in the Tyr kinase domain. RET knockout mice have defects in kidney and enteric nervous system, and the pattern of RET expression in the developing mouse is consistent with the clinical manifestations of MEN2A and MEN2B. [143,144]

## TRANSFORMING GROWTH FACTORS AND THEIR RECEPTORS

TGF-b is the prototype of a family that includes activins and inhibins, bone morphogenic proteins, and müllerian-inhibiting substance whose transmembrane receptors contain an intrinsic serine-threonine kinase. [145] These peptides have membrane-bound receptors that are distinct from other growth factor receptors in that they have serine-threonine kinase activity. TGF-b is the prototypic multifunctional peptide growth factor, and TGF-b mediates wound healing, angiogenesis, and inflammation.[145,146] and [147] TGFs also regulate cellular proliferation, stimulating or inhibiting proliferation depending on the cell type. [145,146,147,148,149,150,151] and [152]

At least five separate forms of TGF-b (TGF-b1 through TGF-b5) have been identified. [153,154,155] and [156] TGF-b1 was initially purified from sarcomas and TGF-b2 was purified from various sources, including a human glioblastoma cell line. The multiple forms of TGFs suggest distinct regulatory mechanisms for expression and may allow a cell using this molecule to generate complex and variable signals, depending on the context of its expression. Inhibins and activins decrease follicle-stimulating hormone secretion from the pituitary, müllerian inhibitory substances affect sexual dimorphism, decapentaplegic gene complex functions as a pattern gene in *Drosophila*, and bone morphogenic proteins may represent the mammalian counterpart of decapentaplegic gene complex. TGFs are secreted in an inactive or latent form that cannot bind receptors. [157,158] and [159] Proteolytic cleavage or acid pH releases an active TGF-b dimer from the latent complex. [160]

Ligand binding studies have found that TGF-b binds three receptors: TGF-b-RI, -RII, and -RIII. Genetic studies indicate that TGF-b-RIII is not involved in the transduction of signals that inhibit growth, but it may sequester or clear bioactive TGF-b. [161,162,163,164] and [165] TGF-b-RII has an intrinsic serine-threonine kinase activity and exists as a dimer on the cell surface. In the absence of TGF-b-RII, TGF-b-RI cannot bind ligand. On binding ligand, TGF-b-RII recruits TGF-b-RI into the hetero-oligomeric structure, and the intrinsic kinase activity of TGF-b-RII phosphorylates a serine residue on a domain in TGF-b-RI called the *GS box*, leading to intracellular signal transduction. Because TGF-b-RI only binds ligand in the presence of TGF-b-RII, TGF-b-RI may also be viewed as the first substrate in the ligand-receptor signal transduction pathway. [166,167] and [168]

Smads were first identified in *Drosophila* (Mad = mothers against dpp) and *C. elegans* (SMA-2) and are the intracellular signaling intermediaries of the TGF-b-RI and TGF-b-RII signal transduction path. There are nine Smads that fall into three classes based on sequence similarity and function. Class I Smads (Smad1, 2, 3, and 5) and the class II Smad (Smad 4) are directly phosphorylated by TGF-b-RI. Upon phosphorylation, class I Smads translocate to the nucleus. Class II Smads may associate with Class I Smads to move to the nucleus. In the nucleus, either alone or complexed with other transcription factors, Smads regulate gene expression. Class III Smads (Smad6, 7) may act as negative regulators of the TGF-b signaling path. [168,169] The tumor-suppressing activity of the retinoblastoma protein and the growth-inhibiting activity of TGF-b1 appear to function through a common pathway. [170,171] and [172] Studies indicate that activation of the TGF-b signal transduction stimulates phosphorylation of cell-cycle cyclin-cdk complexes and induces p16, an inhibitor of these complexes. [172] “Crosstalk” among the TGF-b signaling path and other intracellular signaling paths, such as the ras and MAP kinase paths, may modulate functional responses to TGF-b in some cell types.

## TRANSFORMING GROWTH FACTOR b AND CANCER

TGF-b was initially identified by its ability to induce anchorage-independent growth in rodent fibroblast cell lines. In many human tumors, however, it is a failure to respond to the normal growth-inhibiting signal of TGF-b that may be a factor in the loss of growth control. Now there is strong evidence that TGF-b suppresses the growth of normal epithelial and lymphoid cells. [168,169,173,174,175] and [176] Aberrant TGF-b-RII genes have been identified in a large number of gastric carcinomas. [177] Alterations in TGF-b processing or in its signal transduction pathway may also contribute to the tumorigenic phenotype. A human lung carcinoma cell line, A549, only produces a latent, inactive form of TGF-b, but cell growth can be inhibited if treated with active TGF-b. [178] Further evidence supporting a role for TGF-b as a growth suppressor in human tumors comes from studies in which the anti-proliferative effects of antiestrogens on human breast cancer cell lines [179] or retinoids on promyelocytic leukemia cell lines or NB cell lines are associated with increases in TGF-b secretion and frequently with upregulation of its receptors. [180,181]

Ewing's sarcoma and related peripheral neuroectodermal tumors carry recurrent translocations involving the EWS gene on chromosome 11 to a member of the ets transcription factor family, most commonly FLI1 but also ERG, ETV1, E1A-F, or FEV on chromosome 22. Although the normal ets family genes stimulate TGF-b-RII transcription, the EWS–FLI fusion proteins suppress TGF-b-RII expression. Ewing's sarcoma tumors and cell lines express relatively low levels of TGF-b-RII, and reconstitution of the TGF-b–signaling path in Ewing's sarcoma cell lines suppresses tumor cell growth. This indicates that TGF-b-RII is a target of the EWS-ets fusion proteins and may be important in the genesis of Ewing's sarcoma.[182,183] In NB tumors, low levels of TGF-b-RIII have been reported in advance-stage NB tumors.[184] TGF-b is a potent regulator of bone formation, and, in a sampling of osteosarcoma, tumors that expressed high levels of TGF-b3 had a significant decrease in disease-free survival. [185]

Mutations in Smads have been identified in human cancers. The candidate tumor suppressor gene DPC4, which show loss of heterozygosity in almost 50% of pancreatic cancers, was found to encode Smad4.[186] A subset of sporadic colorectal cancers have mutations in Smad2. [187] Thus, loss of the TGF-b signal transduction path may be an important feature in the genesis of many types of human cancers.

## CELL CYCLE

Extracellular growth stimulating and inhibiting signals converge on a set of evolutionarily conserved enzymes that drive cell cycle progression. The cell cycle is functionally divided into four components; the DNA synthesis or S phase in which DNA is replicated, the mitotic or M phase in which duplicated chromosomes segregate and cytokinesis occurs, the first gap or $G_1$ phase between the end of M phase and the beginning of the S phase, and the second gap or $G_2$ phase between the end of DNA replication and the initiation of cytokinesis ( Fig. 5-1). Although the length of the S, $G_2$, and M phases are essentially comparable among cells, the length of $G_1$ is quite variable and accounts for the varying lengths in cell cycle doubling times for different cell types. A cell is said to be in $G_0$ if it is quiescent and withdraws from the cell cycle either permanently, as in the case of differentiated cells, or for an extended period, as in the case of memory B cells.

Two classes of proteins, cyclins and cyclin-dependent kinases (cdks, cdc), form complexes that serve as an engine driving the progression of the cell division cycle via sequential phosphorylation of target proteins. The cyclin-dependent kinase inhibitors (CKIs) regulate the activity of cyclin-dependent kinases and serve essentially

as a braking system for the cell cycle machinery. CKIs include members of the CIP/KIP family (p21, p27) and members of the INK4 family (p15, p16, p18, and p19). Although the steady-state levels of the various cdks and cdc are constant throughout the cell cycle, transcriptional and post-translational mechanisms variably regulate the levels of cyclin proteins at different stages of the cell cycle. Growth factors activate signal transduction paths, initiating a signaling cascade involving the ras and MAP kinase paths that cause increases in early response genes such as myc, fos, and jun that stimulate cyclin D expression. Cyclins D1 to 3 form complexes with cdk4, cdk6 and are active early in $G_1$. Cyclin E forms complexes with cdk2, is active in late $G_1$, and is considered essential for entry into S. Cyclin A expression peaks in S and forms complexes with cdk2, regulating cell cycle progression through S phase; whereas in $G_2$, cyclin A complexes with cdc2. Cyclin B expression peaks in M and forms complexes with cdc2 regulating the complex series of events needed for mitosis. To ensure that cyclins are present at a particular stage of the cell cycle, they are targeted for proteolysis by ubiquitin and the proteosome pathway. [188,189 and 190]

Cells require exposure to growth stimulatory factors only during the first two-thirds of $G_1$ during which time they become competent for DNA synthesis and no longer require growth factors for cell cycle progression. Cells that do not receive sufficient growth factor stimulation during this time, termed the *restriction* (R) *point*, exit from the cell cycle and either return to $G_0$ or commit to a differentiated state. The retinoblastoma protein, pRB, serves as the molecular switch controlling the $G_1$ R point transition.[191] Growth factor stimulation during $G_1$ leads to increases in cyclin D/cdk4 and cyclin D/cdk6 kinase activity followed by increases in cyclin E/cdk2 kinase activity that sequentially phosphorylate and inactivate pRB, thus enabling cell cycle progression. In the unphosphorylated state, pRB is active and binds a series of transcription factors (E2F1 to 5) preventing them from regulating genes needed for DNA synthesis such as thymidine kinase, DNAa polymerase, cyclin A, B, and cdc2. Although active pRB (hypophosphorylated) can bind E2F that is bound to the promoter regions of genes, Rb is thought to prevent gene transcription by recruiting co-repressors that have a histone deacetylase (HDAC) activity that causes a closed chromatin conformation and prevents access of RNA polymerase to the promoter regions of E2F target genes.[192,193]

Growth inhibitory signals, such as serum starvation or TGF-b, negatively regulate the cyclin-dependent kinase activity and impede cell cycle progression. Withdrawal of serum before the R point causes a decrease in cyclin D and an increase in the cdk inhibitor p27, which binds and inhibits cyclin E–dependent kinases causing a $G_1$ arrest. Activation of the TGF-b signal transduction path increases expression of the cdk inhibitor $p15^{INK4B}$. If $p15^{INK4B}$ binds cyclin D/cdk4 and cdk6 kinases and inactivates these kinases, cells arrest at the R point in $G_1$. If a cell has passed the R point, TGF-b cannot inhibit cell cycle progression. The effective concentrations of cdk inhibitors and active cyclin-dependent kinases determine whether a cell becomes competent to transit the $G_1$/S restriction point.

Another mechanism influencing cell cycle progression is control of genomic integrity. Checkpoints during $G_1$ and $G_2$ ensure that damaged cells or improperly replicated cells are either repaired or exit the cell cycle. Damage to the genome leads to the activation of ATM/ATR and DNA PK family of protein kinases that stabilize p53 levels. p53 levels increase and translocate to the nucleus where, as a transcription factor, p53 stimulates transcription of p21, a cdk inhibitor that inhibits cyclin-dependent kinases. p21 acts on both $G_1$ and $G_2$ cyclin-dependent kinases. Cells arrest until damage is repaired, or, if damage is irreparable, cells undergo apoptosis.[191]

**Cell Cycle Machinery and Cancer**

The loss of both alleles of the RB gene defined RB as the prototypic tumor suppressor gene in retinoblastoma. However in other cancers loss of RB function may be through point mutations, in consensus phosphorylation sites, and/or in the “RB pocket,” which binds the E2F transcription factors. In addition to retinoblastoma, osteosarcoma, Burkitt's lymphoma, and glioblastoma also have mutations in RB.[194]

The CDKN2A gene, which is frequently mutated in human cancer, gives rise to $p16^{INK4A}$ as well as $p16b^{ARF}$. Deletions, mutations, and transcriptional silencing are three mechanisms by which tumor cells functionally inactivate CDKN2A. Almost 100% of glioblastomas do not express p16 either through gene deletion or transcriptional silencing, whereas 65% of astrocytomas have mutations of deletions of CDKN2A.[195] In other pediatric solid tumors, reports of p16 loss vary, perhaps owing to the small sample populations analyzed in each study. Thirty-two percent of NB tumors have LOH at 9p involving the CDKN2A locus. Alterations in the p16 path may contribute to the progression of NB, as 72% of NB cell lines fail to express p16, with 40% of these having methylated promoters.[196] In a small analysis, five rhabdomyosarcoma cell lines and 3 of 12 tumors had homozygous deletions of CDKN2A (p16) and CDKN2B (p15) but not CDKN2C (p18).[197] Thirty percent of Ewing's sarcoma tumors and almost 50% of cell lines showed loss of p16 expression.[198] Deletions of the p15, p16 gene are one of the most common alterations in pediatric acute lymphocytic leukemia.[199]

Genetic alterations in CKI $p27^{kip}$ are rare. Low levels of $p27^{kip}$ expression were associated with a poor prognosis and a shorter median survival compared to aggressive colorectal carcinomas[200] and in breast cancer, prostate, non-Hodgkin's lymphoma, and lung cancer.[201] Systematic studies of p27 levels have not been reported for pediatric tumors.

**Therapeutic Strategies Aimed at Growth Factors, the Cell Cycle Machinery, and Their Signal Transduction Pathways**

As the participants in growth stimulatory signal transduction pathways have been defined, strategies to target and block the action of these proteins has led to the development of a new array of anticancer drugs. Similarly, drugs targeted at potentiating growth inhibitory or suppressing pathways have tremendous therapeutic potential (Table 5-3). Interruption of ligand-receptor interaction can be accomplished by ligand antagonists or antibodies against ligands or receptors. Peptide antagonists have been difficult to develop, but naturally occurring binding proteins, such as IBP-3, that binds IGFs can inhibit peptide activity.[56] Suramin is a polysulfonated napthylurea that interferes with the biologic activities of PDGF, FGF, TGFs, EGF, and IGFs.[202,203,204,205 and 206] Suramin is undergoing clinical phase I and phase II evaluations, and antitumor activity has been seen in treating prostate carcinoma, although the mechanism of antitumor activity is unknown. Although suramin has many activities that are unrelated to its ability to block growth factor activity, drugs based on suramin's chemical structure may be designed to specifically block growth factor activity. Pentosan polysulfate is a polyanionic compound that binds heparin-binding growth factors and has been shown to inhibit tumor cell growth *in vitro*.[207] Extensive preclinical studies identified antibodies to the EGFR or IGFR that are active in inhibiting growth of tumor xenografts.[30,90] Antibodies (Trastuzumab) to EGFR are active in breast cancer, especially in combination with chemotherapy.[32] Receptor-specific antibodies conjugated to radioisotopes have been used for imaging and targeted radiotherapy, and antibodies conjugated to recombinant toxins have tumoricidal activity.

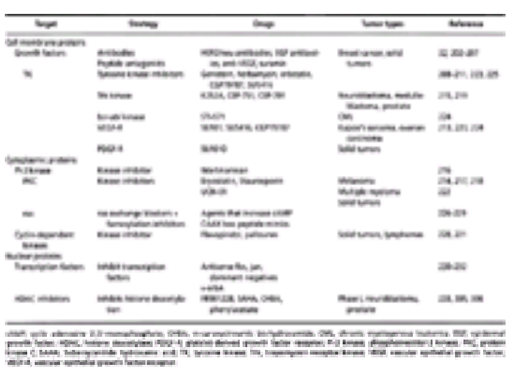

**TABLE 5-3. TARGETING SIGNAL TRANSDUCTION PATHWAYS**

Tyr kinase inhibitors target events at the initiation of signal transduction.[208] Natural products, such as genistein,[209,210 and 211] herbimycin,[212] and bryostatin[213,214] inhibit kinase activity. Genistein inhibits adenosine triphosphate interaction with Tyr kinase domains. Herbimycin A and simple benzoquinones inhibit Src, Bcr-Abl, EGFR, and HER2/neu Tyr kinase activity, presumably by targeting proteins for degradation. Some inhibitors selectively inhibit specific receptors, suggesting selective interference with receptor substrate interactions. For example, a K252A derivative selectively inhibits Trk but not EGFR Tyr kinase activity,[136,215] and wortmannin inhibits PI-3 kinase activity.[216] CGP 53716 inhibits PDGFR autophosphorylation, cellular Tyr, and c- *fos* induction but not similar activities stimulated by EGFR, IGF-1R, or FGFR. This compound also inhibits the growth of v-sis–transformed cells in *in vivo* xenograft models, but not tumors overexpressing EGFR.[217] Bryostatin is a natural product isolated from a marine organism that activates PKC and has broad antitumor activity *in vitro*. A number of these compounds are in phase I testing

(Table 5-3).

Many proteins reside in the cell membrane after posttranslational modifications involving myristoylation (i.e., addition of N-myristyl), palmitylation (i.e., addition of S-palmityl), or prenylation (i.e., addition of geranylgeranyl or farnesyl). [225,226 and 227] An exciting new group of drugs that interfere with protein lipidation have antitumor activity. Prenylation can occur on a protein containing a motif called the *CAAX box*, in which a cysteine is the fourth amino acid from the carboxyl terminus. The ras gene contains a CAAX box and requires farnesylation to function. The finding that oncogenic ras proteins lose their transforming capacity on deletion of the CAAX box led to the development of drugs that could inhibit farnesylation. These drugs mimic the CAAX peptide and, by inhibiting farnesyl transferase, they can inhibit tumor cell growth. The drugs have little activity against normal cell lines *in vitro* but can inhibit tumor growth in animal models.[225,226,227,228 and 229]

The ultimate convergence of signal transduction pathways is to activate transcription factors that regulate gene expression. Antisense nucleotide strategies targeted to promoters or coding regions of transcription factors have been explored primarily in *in vitro* studies.[230] Mutant proteins that interfere with wild-type proteins are called *dominant negative proteins*. One of the first described was v-erb, which produced a mutant thyroid hormone receptor that was capable of blocking the normal thyroid hormone receptor.[231] Dominant negative proteins of known transcription factors can block signal transduction at the level of gene transcription. [232,233]

A direct cdk inhibitor, flavopiridol [220] finished phase I clinical trials in adults with some activity in kidney cancer patients and limited toxicity. Flavopiridol in a phase II trial in patients with metastatic renal carcinoma had a 6% response rate, yet was considered ineffective at the dose and schedule administered. [221] Based on insights from the activity pattern of flavopiridol, the National Cancer Institute drug screen identified paullones, a novel class of small molecule inhibitors of cyclin-dependent kinases.[234] Because cyclin-dependent kinases are key to cell proliferation, the cyclin-cdk or -cdc interaction sites are being analyzed to rationally design drugs that specifically target this site and the protein's kinase activity. [235]

## NUCLEAR HORMONE SIGNAL

### Transduction Pathway

During development, the differentiation and function of many cell types is influenced by hormones, vitamins, and their metabolites and fatty acids. These molecules have nuclear or cytoplasmic receptors, as they do not require surface receptors to transverse the plasma membrane. The nuclear hormone receptors have a modular protein organization: a ligand-binding domain, a DNA-binding region that recognizes target sequences called *hormone-responsive elements*, a receptor dimerization domain, and a transactivation domain termed *AF2* whose protein interactions change upon ligand binding. [236,237] The hormone receptors for retinoic acid, vitamin $D_3$, thyroid hormone, and fatty acids use the retinoid X receptor (RXR) as a heterodimeric partner and bind direct repeat DNA sequences separated by one to five nucleotides (Fig. 5-4 and Fig. 5-5). These receptors bind to DNA in the absence of ligand and may act as repressors of gene transcription. Transcriptional control can act at several levels. Hormone receptors can interact directly with the basal transcriptional apparatus to control the rate of initiation by RNA polymerase II. Another level of regulation occurs through modifying local chromatin structure, thus controlling access of transcription components to the promoter sequence. Recently, a number of transcriptional regulatory molecules called *co-activators* (SRC, ACTR, pCIP, PCAF ) and *co-repressors* (mSin3A, mSin3B, N-CoR) have been found to bind hormone receptors. In the presence of ligand, co-activators bind to the AF2 region of hormone receptors. Co-activators, such as SRC-1 and ACTR, contain an intrinsic histone deacetylation (HAT) activity, whereas other co-activators may bind proteins that contain HAT activity. For some hormone-responsive genes, Creb-binding protein/p300 is recruited to the promoter by binding to the co-activator SRC-1. The ligand-bound nuclear receptor, co-activator, and CPB/p300 complex associate with RNA pol II and with several other accessory factors, to acetylate histones. This leads to a more open chromatin structure which promotes gene transcription (Fig. 5-5A). In the absence of retinoic acid, a co-repressor, N-Cor, binds nuclear hormone receptors, replacing SRC-1 and the associated Creb-binding protein/p300 and RNA polymerase II complex. N-Cor recruits mSin3 and HD1, an HDAC, which leads to deacetylated histones, chromatin condensation, and repression of transcription (Fig. 5-5B). Thus, in the presence of ligand, nuclear receptors switch from binding a protein co-repressor complex containing HDAC activity, which represses gene transcription, to a co-activator protein complex that contains HAT activity and activates transcription. [238]

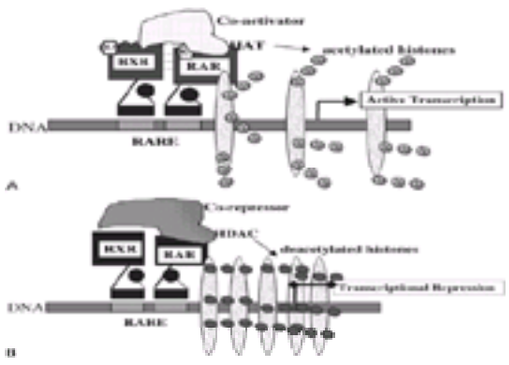


**FIGURE 5-5.** Transcriptional activities of nuclear hormone receptors. **A:** Nuclear hormone receptors upon binding ligand cause an allosteric change in receptor interaction that enables binding of co-activator molecules (such as SRC, ACTR) that may possess an intrinsic histone acetyltransferase activity (HAT) or bind other proteins that contain HAT activity. Acetylated histones lead to an open chromatin conformation that enables access of the basal transcription machinery and RNA polymerase II to the promoter regions. **B:** In the absence of ligand, nuclear receptors are bound by proteins called *co-repressors* (N-CoR, SMRT) that contain an intrinsic histone deacetylase activity (HDAC) or bind other proteins that contain HDAC activity. Deacetylated histones lead to a more closed chromatin conformation and repress transcription. RA, retinoic acid; RAR, retinoic acid receptor; RARE, retinoic acid response element; RXR, retinoid X receptor.

### Retinoids

In the early 1920s, a deficiency in vitamin A, a retinoid derivative, was linked to cancer in rodents. [239] Animals are not capable of de novo synthesis of retinoids, and the primary dietary source is plant carotenoids. Carotenoids in the intestine are converted to retinal and retinol, and retinol is carried through the bloodstream to the liver, where it is stored or distributed by specific binding proteins to various tissues in which it is converted to retinoic acid. [240,241] Intracellular levels of retinoids may be affected by the presence of cytoplasmic retinoid-binding proteins. [242] Vitamin A is necessary for normal epithelial differentiation and limb morphogenesis. [243]

The activities of most retinoids are mediated by nuclear receptors that belong to the steroid hormone receptor superfamily. [244] The DNA sequences of the retinoic acid receptors (RAR)-a, -b, and -g are highly homologous, expressed in a wide variety of tissues, and bind retinoids with various affinities. [245,246 and 247] The RXR class of receptors also contains three subtypes (RXR-a, -b, and -g). The primary RXR ligand is 9- *cis*-retinoic acid.[248] All-*trans*-retinoic acid (ATRA) directly activates RAR and presumably activates RXR through its metabolism to 9-*cis*-retinoic acid.[264] All RAR and RXR subtypes contain multiple isoforms, leading to a great number of combinatorial possibilities by which retinoids may signal, distinguishing them from other hormone or vitamin receptors.

RAR and RXRs bind to DNA sequences called *retinoic acid response elements* that have been found in the promoter regions of the cellular retinoic acid-binding protein II and the RAR-b, laminin B1, and hoxA genes. In some cases, these retinoic acid response elements also contain sequences that are recognized by other transcription factors such as AP-1, a heterodimer of fos, and jun proteins. AP-1 is induced by activation of the PKC signal transduction pathway, growth factors, and the phorbol ester class of tumor promoters.[249] RAR-mediated inhibition of AP-1 activity may be a major mechanism by which retinoids block the action of tumor promoters such as phorbol esters.

### Retinoids and Cancer

The mechanisms by which retinoic acid controls cell growth, suppresses tumorigenicity, and induces differentiation or apoptosis in many tumor types are under investigation.[250] In NB and promyelocytic leukemia cell lines, retinoic acid inhibits growth and induces differentiation ( Fig. 5-6). Among the earliest changes observed in retinoic acid–treated tumor cells is decreased transcription of the oncogenes myc, myb, and cell cycle genes, [251,252 and 253] and induction of TGF-b and its receptors.[180,181] Retinoic acid–inhibition of the growth of the promyelocytic leukemia cell line, HL-60, depends on induction of TGF-b and its receptors and activation of

the TGF-b signal transduction pathway.[180] The differentiation of NB cell lines depends on the ability of retinoic acid to induce the TrkB receptor, and because many NBs constitutively produce the TrkB ligand, BDNF, this leads to the autocrine activation of this signal transduction pathway and neurite extension.[130] Thus, retinoids may exert their effects by activating genes that participate in signal transduction paths involved in the control of cell proliferation and induction of differentiation or apoptosis.

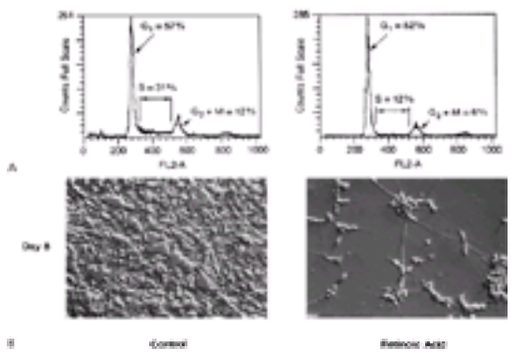

**FIGURE 5-6.** Retinoic acid treatment of a primary culture of cells from neuroblastoma tissue results in a decrease in cell proliferation and induction of morphologic differentiation. **A:** DNA content analysis, using a fluorescence-activated cell scanner of neuroblastoma cells treated with control solvent ( *left panel*) or 5mM RA (*right panel*). A decrease in the percentage of cells in S + $G_2$ + M (18%) is apparent in retinoic acid–treated cells compared with controls (43%). **B:** Dramatic morphologic differentiation induced by retinoic acid in some neuroblastoma cells.

The dermatologic use of retinoids led to studies of epithelial malignancies ( Table 5-4).[254,255] and [256] Isotretinoin (13-*cis*-retinoic acid) was given to patients with advanced squamous cell cancer or preneoplastic lesions, such as keratoacanthomas. Toxic effects included mild joint pain, nausea, headaches, and increases in serum triglycerides. Retinoids are effective in preneoplastic syndromes, such as cervical dysplasia, keratoacanthomas, oral leukoplakia, and papillomas of the bladder.[257,258,259,260,261] and [262] Among patients with xeroderma pigmentosum who developed skin tumors at a high rate, there was a 63% reduction in skin cancers during 13-*cis*-retinoic acid treatment. After cessation of retinoid therapy, the tumor frequency increased 8.5-fold over the previous reduced rate during retinoid therapy. Patients surgically rendered free of head and neck cancer are at high risk for recurrence and developing second tumors. Retinoid therapy reduced the incidence of second primaries (i.e., 12 in controls versus two in isotretinoin-treated patients) but had no effect on primary disease recurrence.[263,264] These studies are consistent with retinoids being effective as chemoprotective agents. A 5-year study of the use of beta-carotene to prevent basal cell and squamous cell cancers of the skin concluded that, in persons with a previous nonmelanoma skin cancer, treatment with beta-carotene does not reduce the occurrence of new skin cancers.[267]

| Tumor | Drug | Toxicity | Parameter | Reference |
|---|---|---|---|---|
| Squamous cell carcinoma | ATRA | Nausea, dry mucous membranes, joint pain, increased triglycerides | 45–83% response | 254, 268, 275 |
| Basal cell carcinoma (xeroderma pigmentosum) | 13-cis-RA | Same as ATRA | 63% reduction in new tumors | 253 |
| Head and neck cancer | 13-cis-RA | Same as ATRA | Prevents second primaries | 252, 254 |
| MDS | 13-cis-RA | Same as ATRA | 25% response | 261 |
| APL | ATRA | Nausea, dry membranes, headache | 67% complete remissions | 267–270, 278 |
| Neuroblastoma | 13-cis-RA | Cheilitis, fissured lips | 22% responses | 279 |
| Cervix | 13-cis-RA + IFN-α | 13-cis-RA toxicities | 50% response | 286 |
| Squamous cell | | Flu-like symptoms, fever, myalgia | 60% response | |
| Neuroblastoma | ATRA + IFN-α | — | Determine activity in selected pediatric solid tumors | 282 |
| Wilms', rhabdomyosarcoma | | | | |
| Neuroblastoma | 13-cis-RA | | Result: increased event-free survival | 283 |
| Solid tumors, breast, colorectal, prostate cancer | Vitamin D | Hypercalcemia | Disease | 295, 296 |
| Liposarcoma, solid tumors | EB1089 | Reduced hypercalcemia | Lipid accumulation in tumor and decrease in cell cycle proliferation | 300, 301 |
| | Troglitazone | — | | |

ATRA, acute promyelocytic leukemia; ATRA, all-trans-retinoic acid; IFN, interferon; MDS, myelodysplastic syndrome; RA, retinoic acid.

**TABLE 5-4. CLINICAL TRIALS USING DIFFERENTIATION AGENTS**

The ability of ATRA and 13-*cis*-retinoic acid to differentiate promyelocytic leukemia cell lines has been studied since 1980. At that time, 13- *cis*-retinoic acid, the only clinically approved retinoid, was beneficial only in isolated cases of patients with acute promyelocytic leukemia (APL).[268,269] A 1988 study reported that ATRA induced complete remissions in 90% of a group of patients with APL,[270] and confirmatory studies also extended this finding to pediatric patients with APL.[271,272] By progressively monitoring bone marrow cells, signs of differentiation were observed in the APL blast cells. APL is highly sensitive to induction chemotherapy with anthracyclines and ATRA. ATRA alone or in combination with chemotherapy improves the disease-free interval compared with chemotherapy alone (complete remission rates range from 72% to 95%) in patients with newly diagnosed APL.[273,274] Retinoid therapy seems to be active in APL patients whose tumor cells contain a t(15:17). The t(15:17) involves the disruption of the RAR-a gene on chromosome 17q21 and the pml (promyelocytic leukemia) zinc-finger transcription factor gene on chromosome 15, leading to a novel chimeric transcription factor, pml-RAR.[275,276] and [277] It is believed that the pharmacologic levels of ATRA relieves the transcriptional repression of the mutant pml-RAR transcription factor by recruiting co-activators with HAT activity to target genes.[278] Retiniod-resistant APL has been found to be sensitive to arsenic trioxide therapy, although the mechanism of arsenic trioxide action is unknown.[279]

In pediatric solid tumors, 13-*cis*-retinoic acid, ATRA, or 9-*cis*-retinoic acid have little activity in phase I trials.[280,281,282] and [283] However, ATRA administered on an intermittent schedule in combination with interferon-a2a has shown limited activity in a Wilms' tumor and NB.[284] For solid tumors, advanced-stage, rapidly progressing disease that has failed conventional therapy may not be the best setting in which to test differentiation agents such as retinoids. To this point, in a randomized trial (CCG-3891) of more than 434 children and adolescents with newly diagnosed Evans stage IV NB, it was shown that patients receiving post-consolidation therapy with high-dose 13-*cis*-retinoic acid achieved 46% 3-year, event-free survival compared to 29% in controls.[285]

## VITAMIN D

### Vitamin D and Its Receptor

Vitamin D is produced in the skin when sunlight or ultraviolet irradiation converts 7-dehydro-cholesterol into a precursor that is transported to the liver by specific binding proteins and processed to the active hormone, 1,25-dihydroxyvitamin D.[286] Vitamin D regulates calcium metabolism and stimulates bone, keratinocyte, and hematopoietic stem cell differentiation. The vitamin D receptors (VDRs) are expressed in a variety of tissues but may be more highly expressed in immature cells such as osteoblasts or crypt cells of the intestinal mucosa.[287,289] A mutation in the DNA-binding domain of VDR occurs in patients with vitamin D–dependent rickets type II, leading to hypocalcemia, rickets, and secondary hyperparathyroidism and elevated levels of vitamin D in plasma. VDR homodimers or heterodimers bind vitamin D response elements, which have been found in the promoters of the osteopontin and osteocalcin genes.[290] Like retinoids, the expression of a number of other genes may be indirectly increased (e.g., calbindins, c-fms, TGF-b, and c- *fos*) or decreased (i.e., c-*myc*, c-myb, colony-stimulating factor, and calcitonin) by vitamin D treatment.

### Vitamin D and Cancer

Vitamin D inhibits the growth and induces differentiation of a number of tumor cell lines *in vitro*. A number of metabolites and synthetic analogs of vitamin D (proposed to be named *deltanoids*) have been developed.[291,292] Clinically useful deltanoids retain their differentiating effects but do not alter $Ca^{2+}$ metabolism. Vitamin D analogs inhibit the growth and induce differentiation of NB,[293] osteosarcoma cell lines,[294] and human breast cancer cell lines. Clinical studies have been limited by hypercalcemia, but stabilization of disease in breast and colorectal cancers has been reported.[295, 296]

## FATTY ACIDS

### Fatty Acids and the Peroxisome Proliferator-Activated Receptors

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that bind fatty acids as well as peroxisome proliferators, a group of chemicals that when fed to rodents cause hepatomegaly, peroxisome proliferation, and an increase in the capacity of peroxisomes to metabolize fatty acids. [297,298] PPARs heterodimerize with RXR and, like other nuclear receptors, bind to specific DNA sequences and activate gene transcription upon ligand activation. There are three PPAR receptors. PPARa is predominantly expressed in hepatocytes, cardiomyocytes, enterocytes, and proximal tubule cells of the kidney. Natural ligands are arachidonic acid–derived eicosanoids, and synthetic ligands include fibrates, drugs that are used in the treatment of hypertriglyceridemia. Activation of PPARa regulates oxidation of fatty acids and detoxification of xenobiotic compounds. PPARg is primarily expressed in adipose tissue and the immune system. PPARg activation drives adipogenesis and the expression of genes involved in the uptake, metabolism, and storage of fatty acids, such as lipoprotein lipase, acyl-coenzyme A synthase, and phosphoenol pyruvate carboxykinase. Natural PPARg ligands are arachidonic metabolites derived from the cyclooxygenase path, whereas synthetic ligands include thiazolidinediones (orally active antidiabetics agents) and certain non-steroidal antiinflammatory drugs. PPARd is ubiquitously expressed and may be involved in metabolism of high-density lipoproteins. Eicosanoids, ethylesters of palmitic and oleic acids are natural ligands of PPARd.

### Peroxisome Proliferator-Activated Receptors and Cancer

PPARg can arrest the growth and induce differentiation in adipocytes, fibroblasts, and muscle cells. [299] PPARg ligands, such as troglitazone, have been shown to inhibit growth or induce apoptosis, or both, in liposarcomas and breast and prostate cancer cells. Troglitazone was found to cause differentiation as assessed by a decrease in lipid accumulation, a more normal histology, and a decrease in a marker of proliferation in the liposarcomas patients [300]; PPARg agonists inhibited the growth of both androgen-dependent and -independent prostate cancer cells, causing a decrease in the production of PSA. Preliminary results from a clinical trial in patients with prostate cancer showed stabilization of PSA levels ( Table 5-5).[301]

| Drug | Trial | Mechanism |
|---|---|---|
| TNP-470 | Phase II: advance adult; phase I: solid tumors, lymphoma, acute leukemias | Inhibits endothelial growth |
| Thalidomide | Phase II: head and neck, ovarian, prostate, Kaposi's sarcoma; phase III: lung, prostate | Unknown |
| Squalamine | Phase II: non-small cell, ovarian; Phase I: advanced cancers | Inhibits sodium-hydrogen exchanger; NHE3 |
| Endostatin | Phase I: solid tumors | Inhibition of endothelial cells |
| SU5416 | Phase III: Kaposi's sarcoma, glioma, advance cancer; phase II: metastatic colorectal | Blocks VEGF signaling |
| SU6668 | Phase I: advanced cancer | Blocks VEGF, FGF, and PDGF signaling |
| Anti-VEGF antibody | Phase I: refractory solid tumors; phase II: renal cell cancer | Monoclonal antibody to VEGF |
| Interferon-α | Phase VIII | Inhibition of bFGF and VEGF production |
| EMD121974 | Phase I: patients with advanced/metastatic cancer | Blocks integrin present on cell surface |
| IFA | Phase I in combination with chemotherapy in solid tumors; Phase II: ovarian | Inhibitor of $Ca^{2+}$ flux |
| Marimastat | Phase II: lung, and breast cancer | Synthetic inhibitor of MMPs |
| Neovastat | Phase II: non-small cell lung cancer | Naturally occurring inhibitor of MMP |
| AG3340 | Phase III: non-small cell lung, hormone refractory prostate cancer; phase II: glioblastoma multiforme | Synthetic inhibitor of MMP |

bFGF, basic fibroblast growth factor; FGF, fibroblast growth factor; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor

From National Cancer Institute (Cancer Topics Web site http://cancernet.nci.nih.gov/newscancertopics.html) with permission.

**TABLE 5-5. ANTI-ANGIOGENIC/METASTASIS THERAPY**

The action of PPARg may be cell type specific or influenced by other cellular alterations, or both. In colon cells, the activation of PPARg has been shown to inhibit growth[302] yet increase polyp formation in an animal model predisposed to colon neoplasia. [303] Furthermore, somatic mutations in PPARg have been detected in 4 of 55 sporadic colon cancers analyzed, and each led to aberrant PPARg function. [304]

## FUTURE THERAPEUTIC DIRECTIONS

The dramatic results achieved with retinoic acid and its associated mild toxicities indicate that differentiation therapy has come of age. With a further understanding of the mechanisms by which these agents work and the nature of the genetic lesions in cancer cells, we should be able to fully integrate differentiation agents into clinical use. Agents that only partially inhibit cell growth may be useful between cycles of chemotherapy if the associated toxicities are minimal and different from those of the cytotoxic drugs. The efficacy of cytotoxic drugs can be enhanced if used in combination with differentiation agents. Agents that induce partial differentiation may be useful if the affected tumor cells express new cell surface proteins that make them more sensitive to innate host defenses or applied immunotherapy. Differentiation agents alone or integrated with biologic response modifiers, chemotherapy, or radiotherapy can significantly amplify the available therapeutic strategies.

The development of nuclear receptor agonists that have better pharmacologic properties than their natural counterparts was the initial step in the development of transcriptionally based therapies. The finding that an inhibitor of histone deacetylation was active in a retinoic acid–resistant APL cell line [278] has led to the development of a number of drugs with similar activities that target transcription at a different level. A group of hybrid polar compounds has been shown to inhibit HDAC. Suberoylanilide hydroxamic acid is capable of significantly suppressing the growth of prostate cancer xenografts in nude mice with little side effects as measured by hematologic anomalies, body weight, and histology[305] and m-caroxycinnamic bis-hydroxamide–induced apoptosis in NB cells.[306]

## ANGIOGENESIS AND METASTASIS

Neovascularization plays a role during initial tumor growth and for the development of metastases. Blood vessels form via two distinct mechanisms: (a) vasculogenesis, which involves the differentiation of endothelial cells from mesodermal precursors., and (b) angiogenesis, which involves the generation of new vessels from preexisting ones. The process of angiogenesis occurs normally in adults during female reproductive cycles; repair, remodeling, or regeneration of tissues; and during wound healing.[307] A number of factors stimulate [e.g., VEGF, angiopoietin-1 (Ang-1), bFGF, PDGF, IGF-1, hepatocyte growth factor, matrix metalloproteinase (MMP), hypoxia-inducing factor, nitric oxide, and Eph ligands] or inhibit [e.g., interferon-a, -b, -g, angiostatin, thrombospondin, tissue inhibitor-2 of MMPs (TIMP-2), heparinases, interleukin-12 (IL-12), endostatin, plasminogen activator inhibitor, and Ang-2] angiogenesis. It is the net tumor cell and micro-environmental balance of these factors that ultimately regulates tumor blood supply. [308,309]

Mechanistically, the angiogenic cascade initiates when the concentration of pro-angiogenic factors in the environment activates receptors [e.g., VEGF interacts with VEGF receptor (VEGFR)] leading to vasodilatation, increases in vascular permeability, and endothelial cell proliferation ( Fig. 5-7). The increase in vascular permeability enables the extravasation of plasma proteins that form a primitive scaffold and degrade basement membrane proteins. Degradation of the basement membrane proteins enables endothelial cells to migrate to the scaffold and become incorporated into the growing vessel sprout. Proteinases of plasminogen activator, MMPs, and heparinases promote angiogenesis by degrading basement membrane proteins, whereas TIMPs interfere with the action of MMPs and plasminogen activator and inhibit angiogenesis. Once a path has been cleared, endothelial cells migrate to the angiogenic stimulus and proliferate, forming the growing vessel sprout.

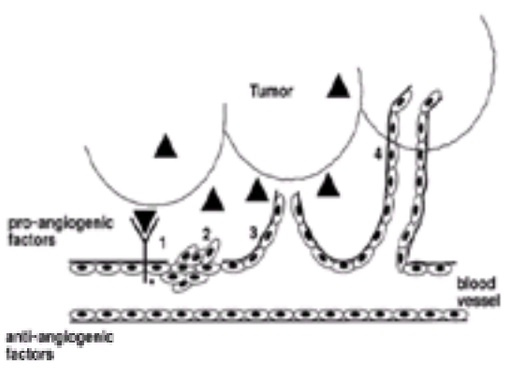

**FIGURE 5-7.** Schematic representation of the critical steps in the angiogenic process. In this model, a pro-angiogenic factor secreted by tumor cells interacts with ligand and activates the receptor tyrosine kinase (1), which leads to vasodilatation, an increase in vascular permeability, and endothelial cell proliferation. This leads to extravasation of proteins that form a primitive scaffold as well as degrade the basement membrane proteins (2). Endothelial cells migrate to the scaffold and form the growing vessel sprout (3), and the elaboration of proteinases enables the endothelial cells to migrate toward the pro-angiogenic stimulus and invade the tumor margins (4).

Metastasis is the process by which a tumor cell leaves its primary environment, enters the circulatory system, survives in the circulation, arrests in the microcirculation of a target organ, extravasates, and begins to grow in a new environment. Both autocrine and paracrine stimuli can provoke the metastatic process. The mechanisms fundamental to the metastatic process are also normal physiologic events that are important during development and tissue renewal. Adhesion, proteolysis, and migration are processes that are used by immune cells during wound healing, neurons during neuritogenesis, neural crest cells during embryogenesis, trophoblasts during endometrial implantation, and, as discussed previously, endothelial cells during angiogenesis. [310]

The first event in the metastatic process is for the tumor cell to lose cell-to-cell adhesive contacts and migrate to a vascular access site. Migration requires cellular motility and the ability to degrade via proteolysis the local stroma and basement membrane proteins. The second key event is vascular access, which requires adhesion to the outside of the vascular basement membrane, local degradation of matrix proteins, and migration through the basement membrane and between endothelial cells to enter the circulation. Once in the circulation, many patterns of dissemination follow circulatory flow, and studies using videomicroscopy have shown that the vast majority of cells arrest in the microcirculation due to capillary restriction. [311] Chemoattractant cytokines may also provide homing signals for metastasizing tumor cells and target tumor cells to various organs. Tumor cells tend to lodge in the first downstream capillary bed and extravasate shortly thereafter. The rate-limiting step in metastasis formation is growth after extravasation. Metastatic tumor cells in their new environment may proliferate and form new tumor foci, remain dormant for extended periods, or die. The processes of cell adhesion, proteolysis, and motility are key to metastatic spread and also play a role in angiogenesis. [310]

Cell adhesion is a process by which cells interact with their environment to facilitate migration or proteolysis and may involve homotypic (e.g., between tumor cells), heterotypic (e.g., between tumor and endothelial cells), and stromal interactions. Adhesion itself consists of attachment, spreading, and, in some cases, detachment. Cell-cell and cell-stromal interactions are mediated by a number of different type of receptors; integrins, laminin receptors, cadherins, cell adhesion molecules, selectins, and CD44. [310]

Proteolysis is the process of degradation of the extracellular matrix proteins of the basement membrane, and it requires the production, release, and activation of a number of different enzymes. Proteolysis is regulated by the presence of proteolytic enzymes as well as by the balance of activators and inhibitors of proteolytic activity. Major proteases and their inhibitors include: (a) plasminogen activators (serine proteases) and SERine protease inhibitors (SERPINS), (b) cathepsins (cysteine proteases) and cathepsin inhibitors, and (c) MMPs (require $Zn^{2+}$ for their activity) and the TIMPs. [310]

Motility is key to the process of metastasis and angiogenesis. Cellular locomotion requires the extension of a pseudopodia or invadapodia, membrane ruffling, attachment to extracellular matrix of the leading edge, and detachment from the extracellular matrix of the trailing edge. The dynamic polymerization and depolymerization of the cytoskeleton and filamentous actin enables the extension of invadapodia. Integrins play a key role in the attachment of invadapodia to the extracellular membrane proteins. Motility can be influenced by the directional movement of a cell toward a concentration gradient of a stimulus (chemotaxis), the random movement of the cell to the stimulus (chemokinesis), or the movement of a cell to an immobilized stimulus (haptotaxis). [310]

Angiogenesis and metastasis are inextricably linked. The following sections delineate some of the proteins that play a role in these processes.

### Vascular Endothelial Growth Factor and Its Receptors

Central to the process of angiogenesis is the family of VEGFs and their Tyr kinase receptors. [312] There are six members of the VEGF family of peptides that are secreted dimeric glycoproteins characterized by eight regularly spaced cysteines that form the cysteine knot; VEGF-B, VEGF-C, VEGF-D, the orf virus VEGF-E, and placenta growth factor (PIGF). VEGF stimulates endothelial cell survival, proliferation, migration, tube formation, and degradation of extracellular membrane proteins in *in vitro* systems while *in vivo* it also regulates vascular permeability. Alternative splicing of VEGF exons leads to $VEGF_{121}$ and $VEGF_{165}$ isoforms, which have different biologic properties. $VEGF_{121}$ does not contain a heparin-binding site and is freely soluble, whereas $VEGF_{165}$ is increasingly more basic and contains a heparin-binding domain that enables it to bind to cell surface heparin sulfate proteoglycans and remain more cell associated.

During development, VEGF is widely expressed and its activity is limited by the spatially restricted endothelial expression of its receptor VEGFR-2. Transcription of VEGF is stimulated by a number of growth factors and cytokines, including PDGF-BB, EGF, TNF, TGFs, and ILs. Tissue oxygen tension tightly regulates VEGF, as hypoxia rapidly and reversibly induces gene expression whereas normoxia decreases VEGF production. In adults, VEGF is expressed in brain, kidney, lung, liver, and spleen. PIGF was derived from placenta and is weakly expressed in lung and thyroid tissues. VEGF-B is most abundant in heart and skeletal muscle; EGF-C is expressed at low levels in the heart, placenta, ovary, small intestine, and the thyroid gland; and VEGF-D is most abundant in lung, heart, and skeletal muscle, the colon, and small intestine. [312]

The VEGFRs, VEGFR-1(Flt-1), VEGFR-2 (KDR/Flk-1), and VEGFR-3 (flt-4), consist of seven Ig-homology domains, a transmembrane sequence, and an intracellular split Tyr kinase domain. The second Ig domain of VEGFR-1 is crucial for VEGF binding, which leads to receptor dimerization and auto- or transphosphorylation. Neuropilin-1, a semaphorin/collapsin receptor involved in axonal guidance, has been identified to bind to some isoforms of VEGF and may enhance VEGFR signaling. In adult tissues, VEGFR-1 is expressed in vascular endothelial cells and binds VEGF, VEGF-B, and PIGF. VEGFR-2 is also expressed on vascular endothelial cells and binds VEGF, VEGF-C, and VEGF-D. VEGFR-3 is mainly expressed on lymphatic endothelium and binds VEGF-C and VEGF-D. [311,312]

### Tie and Eph Receptors

The Tie and Eph receptors are endothelial Tyr kinases that also play a role in the formation and maintenance of the vascular system. There are four known ligands; Ang-1 and Ang-4 bind to Tie-1 whereas there are no known ligands for Tie-2. Ang-2 inhibits Ang-1–dependent signaling. Typically, Tie-1 and -2 are expressed in the vascular endothelium and some hematopoietic progenitor cells. Like VEGFR, inhibition of Tie-2 has also been shown to inhibit angiogenesis and tumor growth. [311,312] and [313]

The Eph receptors are the largest family of Tyr kinase receptors and are thought to play key roles in developmental processes. The ligands of the Eph receptor, are membrane-bound proteins that are divided into two groups; Ephrin-A (EFNA), of which there are currently three identified proteins that are anchored to the membrane by a glycosylphosphatidylinositol link, and ephrin-B (EFNB), of which there are six factors that contain a transmembrane region. Receptors interacting primarily with EFNA are termed *EphA receptors*, whereas those interacting with EFNB are termed *EphB receptors*. Mice lacking EFNB2 and some double mutants lacking the EphB2 and EphB3 receptors die *in utero* due to defects in the vascular system. [314] *In vitro*, EFNB ligands can induce vessel sprouting comparable to Ang-1 and VEGF.

### Cellular Adhesion Receptors

Integrins are a large family of heterodimeric transmembrane cell surface glycoproteins that serve as the major receptors for extracellular matrix proteins and mediate cell adhesion, migration, cytoskeletal structure, angiogenesis, cell survival, proliferation, and differentiation. [315] There are 16 different alpha and eight beta chains that have been identified and a number of variants due to alternate splicing. The alpha-V integrins share a common alpha-V subunit of approximately 150 kd that associates with one of five beta subunits of 115 kd. The alpha-V integrins recognize an RGD amino acid motif that is found on a number of extracellular matrix proteins such as vitronectin, thrombospondin, fibronectin, type IV collagen, laminin, and fibrinogen. Integrin-mediated cell interactions involve interaction with other cell adhesion molecules such as cadherins, intercellular adhesion molecules, and leukocyte-expressed cell adhesion molecules.

Integrins do not possess an intrinsic kinase activity and are linked to the cytoskeleton via a complex of cytoplasmic proteins that includes talin, paxillin, a-actinin, vinculin, and focal adhesion kinase (FAK) ( Fig. 5-1). [316] Paxillin is a multifunctional adaptor protein that can bind intracellular Tyr kinases such as FAK, src, and crk.

The N terminal end of FAK binds to B integrins whereas its C terminal end is involved in focal adhesion targeting and paxillin interactions. The clustering of integrins and adhesion molecules after ligand interaction leads to focal adhesions and activation of FAK and increases in intracellular kinases. Additionally, FAK associates with several different intracellular signaling proteins such as Src-family protein tyrosine kinases, p130Cas, Shc, Grb2, and PI 3-kinase. [317] This enables integrin signaling to activate the ERK and MAP kinase paths as well as the G protein–dependent paths. [318]

Cellular adhesion molecules (CAMs) are transmembrane glycoproteins that are part of the Ig superfamily. These receptors do not possess intrinsic kinase activity, and associated kinase activity has not been reported to date. CAMs have been identified based on their tissue of origin; neural cell adhesion molecules, carcinoembryonic antigen, liver, vascular endothelial, and intercellular adhesion molecules. These molecules are important in mediating homotypic cellular interactions during development and, in the case of neural cell adhesion molecules, are important in neurite extension.

CD44 is a cell surface glycoprotein that also facilitates cell-cell and cell-substrate interactions. CD44 has a role in binding extracellular matrix hyaluronic acid, fibronectin, and collagens, and has a role in the catabolism and production of hyaluronan. [319,320]

Cadherins are transmembrane glycoproteins that mediate cellular interactions that are calcium dependent. Like CAMs there are several cadherins that are tissue related, and they are important in different cell types during development. The most widely studied cadherins are epithelial cadherins or E-cadherins. The extracellular membrane receptor contains a His-Ala-Val sequence and a $Ca^{2+}$-binding region that serves as the interaction site. Like focal adhesions and integrins, cadherins are linked to intracellular kinases of the src family. The intracellular portion of cadherin binds proteins such as a-, b-, and g-catenin and armadillo it to the cytoskeleton via a-actinin. E-cadherin function is frequently disrupted in cancer by alterations in b-catenin and the tumor suppressor adenomatous polyposis of the colon gene (APC), which regulates b-catenin levels. [321] E-cadherin–mediated cell-to-cell interaction results in contact inhibition, and loss of contact inhibition is a hallmark of epithelial tumor cells. Loss of E-cadherin function can occur through mutational inactivation of E-cadherin or b-catenin, transcriptional repression, or enhanced proteolysis. Phosphorylation of b-catenin and E-cadherins by src has been associated with decreased adhesiveness. [322]

**Angiogenesis, Metastasis, and Cancer**

Tumor cells have developed multiple mechanisms to stimulate angiogenesis. Malignant transformation of cultured cells is often accompanied by induction of VEGF. Oncogenic forms of ras and raf and mutated p53 upregulate VEGF mRNA. [323] Tumor cells can also induce stromal elements to produce VEGF. Increased VEGF mRNA expression can also occur in hypoxic conditions associated with uncontrolled cell proliferation. Conversely, tumor cells may also downregulate the production of angiogenesis inhibitors during their transition to the angiogenic phenotype. The net balance of inhibitors and stimulators of angiogenesis is thought to influence angiogenesis and tumor growth. [324]

Increased microvessel density is associated with poor prognosis in a number of cancers. There is an increase in microvessel density in brain tumors with an increasing grade of malignancy, [325,326] and microvessel density and VEGF expression also predict a poor prognostic subgroup in low-grade astrocytoma. [327] In NB tumors, tumor angiogenesis correlates with metastatic disease, Nmyc amplification, and a poor outcome. [328]

CD44 has been determined to be a poor prognostic marker in NB and correlates with Nmyc amplification. [329] In a small study of rhabdomyosarcoma tumors, however, CD44 was expressed in more favorable tumors. [330] Although a rigorous analysis has not been performed in brain tumors, high-grade gliomas expressed CD44 whereas meningiomas, medulloblastomas, and normal brain did not. [331]

The basic tenets of treating solid tumors with anti-angiogenic therapy arise from the observations that solid tumors will not grow beyond a few millimeters without new blood vessels, tumors are partially responsible for stimulating new vessel formation, and the endothelial cells that line the newly formed vessels express molecules distinct from those in mature vessels. Table 5-5 lists a number of clinical trials using anti-angiogenic approaches to cancer treatment. Current clinical studies are in adult patients; however, preclinical studies in pediatrics are promising. TNP-470 has been shown to inhibit the growth of small but not large NB tumors in an animal model. [332]

Clinical metastases present in several patterns: (a) the primary tumor and metastases are present at diagnosis; (b) metastases are present, but the primary tumor is occult; (c) only primary tumor is present at diagnosis but, after removal, metastases appear (within months to years), and (d) metastases disappear after removal of the primary tumor. Folkman has proposed that the majority of the presenting patterns of metastases may be influenced by the balance of angiogenic factors in the milieu of the metastases. [333] Angiostatin, a 38-kd fragment of plasminogen, is a potent anti-angiogenic factor that was identified while studying the ability of a primary tumor mass to inhibit the growth of remote metastases. Excision of the primary tumor led to the growth of distant metastases. This indicated that even though the primary tumor may secrete factors locally that stimulate angiogenesis and support its growth, it may also secrete into the circulation anti-angiogenic factors that suppress the growth of distant metastases. [334] Other anti-angiogenic approaches have centered on blocking pro-angiogenic signal transduction pathways. Antibodies to VEGFR, inhibition of VEGF, and bFGF production by interferons and, finally, blocking the VEGF, FGF, and PDGF kinase activity using small molecule inhibitors, such as SU5416 or SU6668, are in various stages of clinical trials in adult patients ( Table 5-5). [335]

Preclinical studies indicated that TIMPs block tumor cell invasion and metastasis. [336] Furthermore, in a transgenic animal overexpressing TIMP-1 in the liver, SV40 large T antigen–induced hepatocellular carcinoma development was blocked, indicating that proteolysis is important for the process of tumor initiation. [337] This has led to the development of a number of clinical trials using synthetic and natural inhibitors of TIMPs ( Table 5-5). It is unclear whether these approaches alone in humans will be as successful as they are in preclinical models. However such biologic approaches will amplify the number of strategies available to clinicians to use in combination with current cancer treatment modalities.

## APOPTOSIS

Apoptosis is an orderly, programmed, energy-dependent mode of cellular death that occurs in response to a variety of stimuli, including irradiation or chemotherapy, viral infection, growth factor or hormone withdrawal, and cytotoxic lymphocyte killing. Apoptotic responses to some of these stimuli are cell-type dependent. Cells dying by this mechanism exhibit characteristic morphologic (nuclear condensation and fragmentation, cell shrinkage, and relative sparing of the cellular membrane and internal organelles) and biochemical (DNA fragmentation and selected proteolysis) features. [338] Apoptotic cell death is a critical event in normal development and in normal tissue homeostasis, playing a role, for example, in nervous system development and in lymphocyte selection processes in the immune system.

A decrease in apoptosis tendencies also appears to be a key step in the development of tumors. An increase in tissue cell number is a pathognomonic feature of malignant growths, and for many years this abnormal cellular growth was attributed simply to an increase in cellular proliferation and dysregulation of the cell cycle. However, the discovery of the overexpression of the anti-apoptotic bcl-2 gene product in follicular lymphomas [339] raised awareness of the potential importance of decreased cell death in the process of tumor formation. Current concepts now suggest that the increased tissue cell number in tumors arises from a combination of increased cellular proliferation and decreased cellular death. The genetic abnormalities that increase cellular proliferation, such as loss of function of RB or overexpression of c-myc, also lead to increased apoptotic cell death. [340] Thus, for these pro-growth mutations to lead to a net increase in absolute cell number, additional mutations resulting in decreased cell death must occur for tumor growth to occur efficiently. Dysfunction of p53 or overexpression of bcl-2 are examples of genetic changes that could inhibit the death response initiated by c- *myc* overexpression or RB mutation. Subsequent genetic changes in these cells could then contribute to other phenotypic changes associated with tumors, such as invasiveness and metastasis.

In addition to pro-growth genetic changes providing a selection pressure for loss of apoptotic signals during tumor development, the tumor microenvironment can also provide a pressure favoring acquisition of antideath mutations. As the number of cells in a tumor mass grows, significant cellular stresses are usually present, such as hypoxia, intermittent oxidative stress, acid pH, and deprivation of nutrients and glucose. Growth of the tumor mass also results in cellular detachment from normal tissue basement membranes. Such microenvironmental stresses would normally be expected to kill the exposed cells. For a tumor mass to grow beyond a certain size, it must develop mechanisms to survive in such a harsh microenvironment. Similar to the mechanisms a developing tumor uses to survive the growth-promoting stresses discussed previously, mutations in death signaling pathways would allow the developing tumor to survive these microenvironmental stresses. Thus, mutations in survival signaling molecules or gene products involved the apoptosis machinery, and alterations in growth factor or cytokine exposure favors continued development of a tumor mass. Because the goal of anticancer therapies is to selectively kill malignant cells, these selection pressures to acquire antideath mutations also negatively impacts on the success of therapy (discussed in the section Therapy Aimed at Modulating Apoptosis).

## Mechanisms of Apoptosis

Clarification of the molecular events involved in cell death signaling pathways has advanced considerably in the past few years. Although it is almost certainly an oversimplification, there are now considered to be two major types of pathways involved in programmed cell death; those that involve signaling through the mitochondria and those that do not appear to involve mitochondria. As a generalization, stress-induced cell death, such as that initiated by chemotherapy or radiation therapy, involve signaling through mitochondria. The bcl-2 family of proteins are critical components of these signaling pathways. In contrast, cell death initiated by certain cytokines, such as TNF and related proteins, appears to occur by a different signaling pathway. It is noted that both pathways appear to converge on the same effector mechanisms of actually executing the cell through degradation of intracellular macromolecules, such as the DNA ( Fig. 5-8).

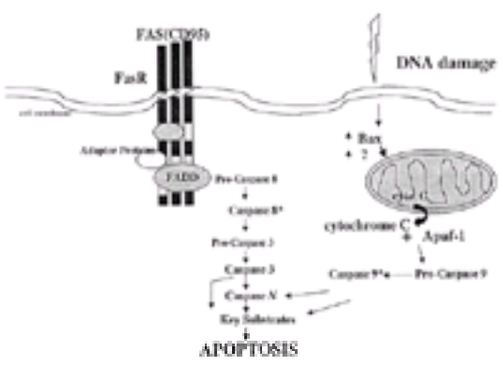


**FIGURE 5-8.** Schematic representation of critical steps in the current models of the two major apoptosis pathways. Cytotoxic stresses, such as that induced by DNA damage, result in the induction of the protein Bax and presumably other gene products. By mechanisms that remain poorly understood, cytochrome C is subsequently released from mitochondria into the cytosol where it associates with Apaf-1 and pro-caspase-9, the latter of which becomes cleaved and activated. Activated caspase-9 then activates caspase-3 and other effector caspases, resulting in destruction of cellular macromolecules, such as DNA, and cell death. The second pathway is initiated by death ligands binding to death receptors, exemplified by the Fas pathway. Binding of ligand to receptor at the cell surface results in recruitment of adaptor proteins (FADD in the case of Fas) that causes cleavage and activation of caspase-8. Activated caspase-8 then activates effector caspases, such as caspase-3, and the terminal phase of apoptosis is similar to that initiated by the cytotoxic pathway. Asterisks represent activation of caspases.

The first insight into control of apoptosis arose from identification of the anti-apoptotic gene, bcl-2, during the molecular characterization of the t(14;18) chromosomal translocation commonly seen in follicular lymphomas.[339] The follicular lymphoma model was that the abnormal lymphoid cells grow initially because of loss of cell death tendencies due to bcl-2 overexpression caused by the translocation of the bcl-2 gene from chromosome 18 to the actively transcribed Ig gene on chromosome 14 in B-lymphoid cells. At this point, such lymphomas are rather indolent, but are difficult to cure with chemotherapy. At some later stage, however, they develop other genetic changes and become more aggressive. A model has been proposed in which the bcl-2–like protein, bax, can drive the cell toward apoptosis when it forms complexes with itself.[341,342] If the bcl-2 protein is expressed at high levels, however, it can form a complex with bax, preventing bax homo-dimerization and inhibiting cell death. A family of other anti-apoptotic proteins with homologies to bcl-2 and bax, such as bcl-$x_L$ and mcl-1, have been identified.[343,344] The list of anti-apoptotic and pro-apoptotic gene products continues to grow, and it is suggested that members of this family of gene products related to bcl-2 interact with each other to influence apoptotic tendencies and that the utilization of a particular family member is a cell-type–dependent process.

Death of the cell during apoptosis is dependent on a series of highly regulated proteolytic cleavages that result in selected activation or inactivation of certain molecules and eventually result in the highly ordered internal destruction of the cell. The critical proteases involved are a family of cysteine-directed proteases, referred to as *caspases*.[345] After genotoxic damage, as would be induced by many chemotherapeutic agents, the release of cytochrome C from the mitochondria and its association with the Apaf-1 protein is a requisite event for cell death. [345,346] This release of cytochrome C appears to be dependent on the appropriate modulation of the activity of bcl-2 family members, which are localized in the mitochondrial membranes. [342,347,348 and 349] Once cytochrome C is released, this complex of cytochrome C and Apaf-1 combines with and initiates the specific cleavage of caspase-9, and activated caspase-9 initiates the cleavage of other specific caspases, such as caspase-3 (Fig. 5-8). These activated caspases then carry out the final steps of macromolecular destruction in the cell.

In addition to this pathway of cellular suicide initiated by genotoxic stress, a group of extracellular molecules can also act as death-inducing signals by interaction with selected cell-surface receptors (called *death receptors*). This process of cell death in this situation occurs via activation of a different caspase-dependent pathway, one that does not appear to be critically dependent on mitochondria ( Fig. 5-8).[342,350] In this scenario, a specific cytokine binds to its cognate receptor and the receptor undergoes a trimerization event and binds to adaptor proteins that ultimately activate caspase-8. This particular caspase does not require cytochrome C or Apaf-1 to activate downstream caspases, such as caspase-3, but the subsequent mechanics of macromolecular destruction can proceed more directly in a manner like that described above for the caspase-9–dependent pathway. Fas-ligand and Fas receptor are examples of an extracellular molecule and death receptor that initiate programmed cell death in this way, and this pathway appears to be particularly important in the regulation of cell death in lymphoid cells. TNF and the recently described TRAIL family[350] are additional examples of cytokines that act via this type of pathway. Although genotoxic stress and Fas- or TNF-induced cell death appears to primarily utilize two different signaling pathways, recent data have suggested that these distinctions are an oversimplification and that cross-talk between these pathways probably occurs.

## Apoptosis Modulation and Human Tumor Development

These apoptotic pathways do not operate in isolation in the cell. Other signaling pathways can influence the activation of one or more steps in apoptosis signaling. For example, growth factors, such as IL-3, which provide survival signals to cells, appear to act via modulation of one or more steps in these pathways. [349,351] One particularly interesting mechanism along these lines is the IL-3–induced phosphorylation of the pro-apoptotic protein, BAD, which causes the release of the anti-apoptotic protein, Bcl-$X_L$, promoting cell survival.[352] It is likely that mutations leading to activation or overexpression of growth factors or growth factor receptors in tumors arise because of their ability to inhibit death signals. This could include overexpression or mutation of EGF, HER2/neu, and IGF-1, all of which have been reported to be abnormal in multiple tumor types, including a number of pediatric malignancies. Similarly, mutation of gene products downstream of these receptor kinases (e.g., activation of Akt), can also be activated in tumors and provide similar anti-apoptotic signals. It is likely that certain chromosomal translocations found in pediatric malignancies fall into this category. It appears that the bcr-abl translocation found in Philadelphia chromosome–positive leukemias contributes to leukemia formation via its role as an anti-apoptotic signal. [353] Similarly, the E2A-HLF translocation found in the highly resistant pro-B cell leukemias appears to provide significant protection from apoptosis.[354] Similar findings are likely to be found in some of the specific translocations seen in sarcomas and other tumors in the pediatric population. Blockade of the survival signals resulting from overexpression or activation of these receptors or from these specific translocations is one potential approach to enhancing the efficacy of tumor therapies (see the section Therapy Aimed at Modulating Apoptosis).

p53 is the most commonly mutated gene in human cancers identified to date[355] and the propensity of human tumors to mutate p53 is likely due to its role in apoptosis signaling. In certain cell types, such as lymphoid cells, p53 is absolutely required for genotoxic stress, such as that induced by ionizing irradiation, to induce rapid apoptotic cell death.[356,357] However, p53 protein is not an integral component of the apoptotic machinery; rather, it signals to the apoptotic machinery. One mechanism by which p53 can influence apoptosis in certain cell types after certain stimuli appears to be via transcriptional induction of the bax protein. [358] However, p53 activation does not induce bax in many cell types and, even under the best of circumstances, bax is only partially responsible for p53-induced apoptosis. [359] It has been recently demonstrated that p53-mediated apoptosis is dependent on both Apaf-1 and caspase-3, [360] but how p53 activates these components of the apoptotic machinery remains unknown. One of major selection pressures for tumors to mutate p53 appears to be because of its critical role in hypoxia-mediated apoptosis. [361] Although the mechanism by which p53 causes apoptosis after hypoxia also remains to be elucidated, it has recently been suggested that it does not even involve transcriptional activation by p53.[362]

Mutations of p53 are relatively common in the majority of carcinomas found in adults, but most pediatric malignancies rarely mutate p53. [363] For example, p53 mutations are rare in acute lymphocytic leukemia, primary AML, and most of the embryonic tumors seen in childhood. However, p53 mutation can be seen in either

resistant variants of some of these tumors or after relapse. For example, p53 mutations rarely occur in T-cell leukemias at diagnosis, but they can be seen in relapsed cases, and these cases have very poor prognoses.[364] Although favorable-histology Wilms' tumor rarely, if ever, has p53 mutations, p53 mutation is associated with anaplastic Wilms' tumor.[365] It should also be noted that the p53 pathway can be inactivated by mechanisms other than p53 mutation, such as mdm2 overexpression or expression of certain viral gene products like human papillomavirus 16 E6.[366] Thus, some tumors can partially or totally inactivate p53 function even without mutating the gene itself. Mdm2 overexpression is found in some cases of osteosarcoma, and human papillomavirus is associated with cervical carcinomas.[367,368]

Mutations in proteins that are integral components of the apoptotic machinery appear to be less common than mutations in these “signaling” gene products. Bcl-2 overexpression occurs in follicular lymphomas and a smattering of other tumor types, but is not a commonly mutated gene in human cancers. Similar statements can be made about other bcl-2 family members, both pro-apoptotic and anti-apoptotic. There is little information implicating alterations in caspases themselves in tumorigenesis. In contrast, death receptor signaling pathways have been intensively studied in human tumors. For example, TNF receptor has been examined in human tumors, and there are many reports implicating Fas in apoptosis signaling after genotoxic stress and examples of alterations in Fas signaling pathways in tumor cells. Finally, the selective absence of “decoy” receptors for the death ligand, TRAIL, on malignant cells has led to significant interest in TRAIL-like compounds as a mechanism to selectively kill tumor cells.[350,369,370] In NB, any strategies aimed at targeting death receptors or compounds whose mechanism of action utilizes death receptors is compromised, as the caspase-8 gene is hypermethylated and not expressed in a number of NB tumors, particularly those that contain amplified Nmyc.[371] However, NB may be sensitive to ligands that activate death receptors if used in combination with compounds that would inhibit methylation or relieve transcriptional repression and induce caspase-8 expression.

### Therapy Aimed at Modulating Apoptosis

A major limiting factor for cancer cures at this time is the toxicity of chemotherapy and radiation therapy to normal tissues. The doses of current anti-neoplastic agents that would be required to kill resistant tumors would also lead to patient mortality. The selection pressures for loss of apoptotic pathways during tumor development only serve to make this selective killing of tumor cells with cytotoxic agents more difficult. Thus, the more we understand about the molecular and cellular differences between tumor cells and normal cells, especially with regard to survival and death signaling pathways, the more likely we will be able to achieve tumor cell selectivity. One can envision two primary ways to enhance tumor cell kill through modulation of apoptosis tendencies: Either the apoptotic machinery or survival signaling pathways could be targeted. Alteration of apoptotic tendencies with either of these approaches could then either be used in combination with cytotoxic agents to enhance tumor cell responses, or it is conceivable that they could enhance tumor cell death on their own without a requirement of an additional apoptosis stimulus.

In general, it is perhaps more difficult to selectively target the apoptotic machinery in tumor cells. If some functional aspects of a bcl-2 family member are altered, then it is likely that normal cells would also be affected. The same could be argued for direct modulation of caspases. However, because it is likely that tumor cells are more dependent on survival signals than normal cells, it is conceivable that direct inhibition of the apoptotic machinery could have a selective effect on tumor cells. As mentioned, there is also already significant interest in modulation of death receptor pathways because there is reason to believe that tumor-cell selectivity could be achieved.[350,369,370]

It is not difficult to envision how inhibition of signaling pathways, alone or in combination with cytotoxic agents, could lead to enhanced and more selective tumor cell kill. Development of a drug that inhibits the bcr-abl Tyr kinase in chronic myelogenous leukemia has already provided the proof-of-principle for selective inhibition of a chimeric protein resulting from a tumor-specific alteration.[372] It is likely that this inhibitor is working in chronic myelogenous leukemia because of its blockade of the anti-apoptotic effects of bcr-abl. Although it appears to have activity used as a single agent, it is quite conceivable that it will be even more effective in combination with cytotoxic agents. This synergy would be expected because the cytotoxics kill tumor cells by engaging the apoptotic machinery, and blockade of survival signals would theoretically make such agents more effective killers of tumor cells. Similar targeting of specific translocations in other tumors, most of which are found in the pediatric population, could similarly result in novel and effective approaches to treatment of a variety of pediatric tumors. Blockade of growth factor receptors, such as EGF, IGF-1, and HER2/neu, has also been shown to have clinical benefit, perhaps most dramatically in combination with other cytotoxic agents. Optimal utility of such inhibition in the treatment of pediatric tumors would require significant characterization of these various survival pathways in these tumors. Although some information exists, more complete characterization would be highly desirable for effective design of clinical trials using such inhibitors.

## SUMMARY

As we continue to learn more about intracellular signaling proteins and the mechanisms by which they regulate decisions of normal and tumor cells to proliferate, differentiate, stimulate angiogenesis, metastasize, or die, it is certain that new and improved therapies will be forthcoming. The complexities and intricacies of these processes may appear daunting, but unifying concepts should emerge. Like the development of multimodality treatments and combination chemotherapy, it may be necessary to use several of these compounds in conjunction, increasing therapeutic options.

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# 6

# TUMOR IMMUNOLOGY AND PEDIATRIC CANCER

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## INTRODUCTION

The human immune system is a complex network of distinct cell types with remarkable and varied functions. Its mission is to maintain the physiologic and functional integrity of an individual by providing an anatomic and functional barrier to invasion by foreign, pathogenic organisms. Its hallmark is the ability to differentiate foreign from self and to remember that difference. An effective immune system eliminates the growth of invading foreign pathogens and prevents their entry when possible. Individuals who have inherited or acquired severe immunodeficiency are susceptible to potentially lethal infections that should have been controlled by the missing component of their immune system. Because fatal infection remains one of the more frequent terminal events for many children and adults who die of cancer or its sequelae, there is an important interface between clinical oncology and immunology.

This chapter delineates the molecular, cellular, and clinical interactions between the immune system and neoplastic disorders. It focuses on the cells of the immune system and their functions, with specific emphasis on abnormalities that are particularly relevant to children with cancer. These include immune dysfunction leading to or found at the time of cancer diagnosis and immune dysfunction occurring as a result of cancer treatment. Of greatest potential importance to the discipline of tumor immunology is the characterization of the two-way interaction between an individual's immune system and the cancer. The growing discipline of cancer immunotherapy is based on the hypothesis that controlled manipulations of the immune mechanisms involved will provide clinically meaningful antitumor therapy. These immunotherapeutic approaches were stimulated by several historical observations, establishing a link of uncertain strength and mechanism between the immune system and the prevention of human tumor growth.

## ANTITUMOR IMMUNITY IN HUMANS: A HISTORICAL PERSPECTIVE

The primary focus of the discipline of tumor immunology has been to characterize the mechanisms whereby immune reactions can recognize neoplastic tissue and to take advantage of this process in the form of preventive or curative therapy. Several sources of historical evidence suggested a potential role for the immune control of tumor growth. Histologic examinations of many tumor types have revealed a ring of inflammatory cells at the tumor's edge, suggesting an ineffective immune response under way against the tumor.[1] Certain patients, particularly those with melanoma or renal cell carcinoma, experienced well described, but rare, spontaneous regression.[2] Similar regressions of residual pediatric neuroblastoma (particularly in infants) were observed more frequently [3] in certain patient groups after surgical resection (see Chapter 31). Occasional “spontaneous” regressions (e.g., in children with leukemia) occurred during concurrent systemic bacterial or viral infections. Thus, some initial approaches at immunotherapy intentionally used bacteria or their toxins to try to induce immunologic tumor regressions; these approaches met with some success, but the mechanisms involved remain in question.[4]

Children with inherited immunodeficiency diseases, organ allograft recipients, and individuals receiving immunosuppressive therapy show high frequencies of certain cancers. A similar increase in the incidence of certain cancers (particularly lymphomas and Kaposi's sarcoma) was later identified in adults with human immunodeficiency virus (HIV)–induced acquired immunodeficiency. These observations suggested to some researchers that an intact immune system might play a strong surveillance role in destroying incipient cancers and thereby protect against development of malignancy. Certain well-studied patients showed that immune reactions could somehow be involved in tumor control. In 1964, Woodruff published the clinical details for a 47-year-old woman who had a solitary, isolated melanoma of the right foot excised with wide margins and no evidence of deep invasion or metastasis. No further therapy was given. [5] Three years later, a breast nodule was found, surgically resected, and found to be adenocarcinoma of the breast, clearly distinct histologically from the prior melanoma. Postoperative radiation therapy was then given to the chest wall and regional nodes. Within 1 month, thousands of subcutaneous melanoma nodules were observed in the irradiation field. Large visceral metastases of melanoma soon developed and proved fatal. This case strongly suggests that host immune mechanisms were keeping melanoma micrometastases in check for 3 years and that the local radiation therapy inhibited those mechanisms, allowing local outgrowth and further spread. Although other hypothetical mechanisms may also (or alternatively) be involved, an immune component seemed to be playing a role.

Another clinical experience argued strongly that an immune reaction could play an important role in controlling or preventing tumor growth. Analysis of data from a variety of bone marrow transplant (BMT) teams suggests an immunologically mediated antileukemic effect can prevent leukemia relapse ( Fig. 6-1) after allogeneic bone marrow transplantation for acute leukemia.[6,7] Three observations were derived from these clinical data. [8] First, leukemia recurrences were observed less frequently in patients who developed immune-mediated graft-versus-host disease (GVHD). Second, more leukemia recurrences developed in the recipients of marrow that had been depleted of T cells. Third, more relapses occurred among the recipients of syngeneic (identical twin) marrow (i.e., no alloantigens would be immunologically recognized) than among HLA-identical siblings (i.e., in whom minor histocompatibility alloantigens could stimulate immune responses), even in the absence of clinically detectable GVHD. These data strongly suggested that T cells from the donor marrow were involved in allorecognition and were also playing a role in destroying microscopic residual leukemia after transplantation, thereby preventing recurrence. Thus, several forms of anecdotal evidence suggest the presence of potentially therapeutic immune reactivity to human tumors. However, converting these observations into meaningful, testable treatment approaches requires a thorough characterization of the components of the immune response that may be involved and the mechanisms by which these responses are regulated.

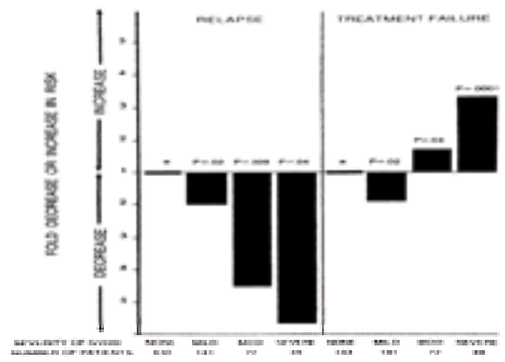


**FIGURE 6-1.** Actuarial probability of relapse after bone marrow transplantation for early leukemia according to the type of graft and development of graft-versus-host disease (GVHD). (From Horowitz MM, Gale RP, Sondel PM, et al. Graft-vs-leukemia reactions following bone marrow transplantation. Blood 1990;75:555, with permission.)

## IMMUNE SYSTEM

Critical examination of the immune system generally requires dividing immune responses into a variety of parts, thus emphasizing distinctions of the various components. Although such models in many ways artificially divide interconnecting members of this elaborate host defense network, they also serve to ease the ability to understand and to study this very complex system. One such division is shown in Figure 6-2, wherein the immune system is divided into “innate” versus “adaptive” immunity. In this model, *innate immune responses* are those that are not antigen specific and for which there is no evidence of immunologic memory, whereas *adaptive responses* refer to the classical, antigen-specific responses carried out by B cells and T cells. Innate immunity comprises responses carried out by neutrophils; phagocytes, including monocytes; and tissue macrophages, eosinophils, natural killer (NK) cells, and the complement cascade. These components generally serve as the first line of defense against infectious pathogens. The central role of innate immune responses in controlling bacterial infection in cancer patients is well known and is reviewed in the Chapter 41. In this chapter, we focus primarily on adaptive responses and their potential role in antitumor immunity.

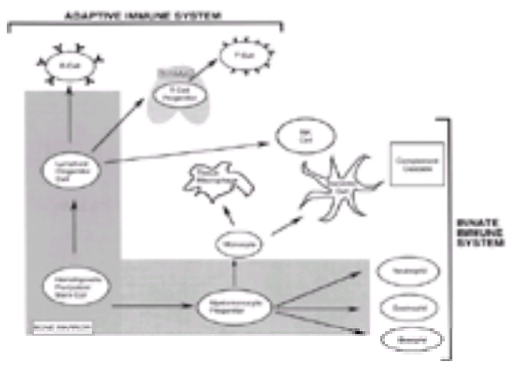


**FIGURE 6-2.** Innate and adaptive immune systems. All cells of the immune system are ultimately derived from pluripotent stem cells. These give rise to two distinct progenitors, one for lymphoid cells and the other for myeloid cells. The common lymphoid progenitor has the potential to differentiate into T, B, or natural killer (NK) cells, depending on the microenvironment to which it homes (i.e., the thymus or bone marrow). Immune cells derived from primitive myeloid progenitors include phagocytic effectors (e.g., macrophages, neutrophils, and monocytes) as well as dendritic cell populations. It should be emphasized that the distinction between these systems is somewhat artificial because there is significant crosstalk between innate and adaptive immune effectors during the initiation and completion of the immune response.

More recent research has shown that cells contained within the innate immune response network play important roles in initiating, amplifying, and potentially directing adaptive immune responses, thus emphasizing the interconnectedness of the entire system. For example, interleukin-12 (IL-12) produced by activated monocytes in response to a variety of stimuli induces interferon (IFN)-g production by NK cells, which then serves to direct T-cell responses toward a T helper 1 (Th1) phenotype.[9] A second example is the evolving role of antigen-presenting cells (APCs) in immune response induction. It is now clear that APCs can be derived from monocyte populations as a result of exposure to a variety of inflammatory stimuli.[10] These cells then serve to initiate adaptive immune responses by providing critical costimulation for T cells. Finally, in the later stages of the adaptive immune response, T cells and B cells recruit a variety of effector cells and molecules typically relegated as components of the innate immune system to carry out immune responses. One example of this is the role that can be played by eosinophils in eradicating tumors, which was recently shown in a tumor transplant model.[11] Therefore, although this chapter emphasizes the basic biology of T and B cells and the mechanisms by which their adaptive immune responses may relate to antitumor immunity, other leukocytes (e.g., NK cells, dendritic cells, and monocytes) that directly or indirectly contribute to antitumor immunity are discussed as well.

### T Lymphocytes

T lymphocytes (designated *T* for their thymic origin) function primarily to eradicate viral infections. Therefore, these cells evolved primarily to optimize their capacity to recognize and eradicate infected cells based on the recognition of intracellularly produced viral products. In modern medicine, however, T-cell responses are also important for their central role in autoimmunity, rejection of transplanted tissues, and potential for antitumor responses. Clearly, many of those features that render T cells efficient for recognizing and eradicating virally infected cells are those same features that are central to the generation of antitumor immune responses. In this section, we review currently accepted models for T-cell recognition, activation, and effector function as a basis for framing discussions of the theory behind immune-based antitumor strategies discussed later in this chapter.

#### *Role of the T-Cell Receptor in the Generation of T-Cell Receptor Diversity*

The first of the unique characteristics of T cells is the fine specificity of antigen recognition by the T-cell receptor (TCR) complex. T cells are defined by the presence of the TCR complex, which is comprised of the invariant CD3 molecule associated in most T cells with the ab heterodimer.[12] The CD3 component of this complex (Fig. 6-3) is a multimolecular cell-surface glycoprotein (comprised of g, d, e, and z chains) that is responsible for transmitting activation signals to the interior of T cells after interaction with antigen. This is accomplished via activation of protein kinase C and release of intracellular calcium stores via activation of phosphatidylinositol-3 kinase, which generate a series of intracellular tyrosine phosphorylation signal–based events, eventually resulting in cell division and cytokine production. The CD3 molecule is identical on all human T cells. Therefore, although the CD3 portion of the TCR is absolutely essential for transmitting activation signals, it plays no role in endowing T cells with the very high level of antigen specificity for which they are very well known.

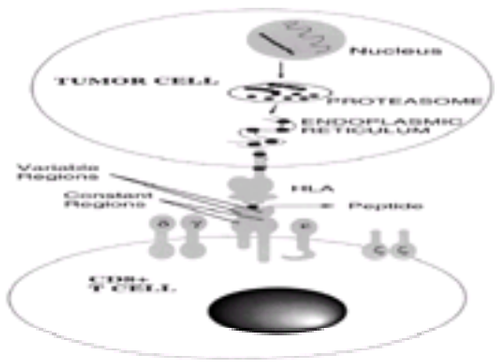


**FIGURE 6-3.** Peptide recognition by the T-cell receptor (TCR) complex. The a and b polypeptide chains of the TCR form a heterodimer anchored in the T-cell membrane. The heterodimer recognizes and binds to peptide associated with an HLA molecule on the surface of a presenting cell (shown in the figure as a tumor cell). The nonpolymorphic CD3 polypeptides (g, d, e, and z) are assembled together with the abTCR and are involved in signal transduction. Peptides presented by HLA class I molecules are typically derived from the intracellular processing of endogenous proteins that are degraded in the proteasome and subsequently are transported to the endoplasmic reticulum where they bind nascent major histocompatibility complex molecules before transport to the cell surface. (Adapted from Krensky AM, Weiss A, Crabtree G, Davis M, Parham P. T lymphocyte-antigen interactions in transplant rejection. N Engl J Med 1990;322:510.)

Rather, for most T cells, the fine specificity of the immune response to individual antigens is endowed by the abTCR heterodimer. Indeed, studies have shown that peptides that differ in only one amino acid can show widely disparate activation potential for a given abTCR. [13] This 95-kd molecule is expressed on the cell surface in close association with the CD3 complex. Its expression is controlled by genes, which are similar in structure to immunoglobulin (Ig) genes. [14] The germline cells and nonimmune cells of a given individual all contain an identical pattern of abTCR genes maintained in germline configuration, which include a linear arrangement of segments termed *variable* (V), *diversity* (for b chain only) (D), *junctional* (J), and *constant* (C) regions. The TCR genes for both the a chain and b chain of the abTCR undergo rearrangement during T-cell differentiation within the thymus, resulting in estimated $10^{12}$ different possible combinations. This combinatorial diversity is then further extended by posttranslational modifications of the abTCR (N segment additions), which yield an enormous variety of TCRs (estimated $10^{15}$), which can potentially exist within any individual. Importantly, after the process of TCR rearrangement is completed within the thymus, each individual T cell expresses only one form of the rearranged abTCR, and all subsequent progeny of this T cell also express the identical rearranged abTCR.

From this enormous T cell repertoire, a highly sophisticated series of positive and negative selection events occurs within the thymus, resulting in the survival of T cells bearing only a mere fraction of the abTCR specificities that are possible in any given individual. Based on current concepts, those T cells that survive thymic selection are those bearing TCR specificities that show enough affinity for self-major histocompatibility complex (MHC) molecules to be positively selected but show a relatively low affinity toward antigens expressed within the thymus, as these TCRs are predicted to be able to recognize foreign pathogens in the context of self-MHC but have a low probability of inducing autoimmunity (reviewed in ref. 15).

Molecular analysis of the TCR DNA within T-cell populations can distinguish individual T-cell clones from one another by their unique TCR DNA gene rearrangement patterns. Such analyses are useful in distinguishing polyclonal reactive processes (i.e., a T-cell response to a viral antigen) from monoclonal malignant expansions (i.e., a T-cell malignancy). Furthermore, the molecular monitoring for the presence of clonally derived malignant T cells in T-cell acute lymphoblastic leukemia and T-cell lymphoma using polymerase chain reaction techniques can allow for the sensitive detection of minimal residual neoplastic disease by amplifying TCR gene patterns that match those expressed on the original leukemia cells (see Chapter 8, Chapter 19, and Chapter 24).

Although most mature T cells use the abTCR, approximately 5% use a less well-characterized gdTCR dimer. [16] The gd genes are similar in structure to the ab genes but have fewer variable segments. The function of these gdTCR–bearing cells remains incompletely characterized. One unique aspect is that gdTCR+ cells generally reside in the gut and do not circulate. It is postulated that these cells contribute to the first line of defense against some infectious pathogens, as they show evidence for cytolytic capacity and recognition of virally infected cells. However, the gdTCR recognition of viral pathogens differs from abTCR recognition in that it is not virus specific (reviewed in ref. 17).

In summary, TCR gene rearrangement occurs early in ontogeny and gives rise to a diverse repertoire of T cells that are poised to respond to the enormous variety of antigens that are encountered throughout the lifetime of the host. On encounter with foreign or potentially dangerous antigens, a highly sophisticated surveillance network serves to ensure that even rare T cells bearing appropriate abTCR specificities will come into contact with antigen to which they can respond, leading to amplification of clones with appropriate TCR specificities. This model, now termed the *clonal selection theory*, was initially postulated in 1900 by Ehrlich and has been shown to be the basis for both T-cell and B-cell arms of the adaptive immune response. Clearly, the appreciation of the fine specificity of the TCR is one feature of T-cell immunity that could potentially be exploited for antitumor effects as it gives rise to the prediction that even minor point mutations in tumor-specific or tumor-associated molecules could potentially be recognized by TCRs and form the basis for an antitumor immune response.

### *Effector Functions of T Cells*

T cells activated to participate in immune responses mediate their immunologic roles through two distinct pathways. One involves direct killing of other cells through direct contact with the target cell that is to be destroyed. The second involves inducing other cells to undergo specific changes, which are dictated by the soluble molecules released by the activated T cell. In general, these distinct functions are performed by distinct subpopulations of T cells.

The cytotoxic T cells (CTLs) are characterized by their surface expression of the CD8 differentiation marker (a listing of all of the CD molecules referred to in this chapter is included in Table 6-1). These CTLs recognize the target cells that they are about to kill through their T-cell antigen receptor molecules (see the previous section) and then cause rapid cellular destruction by two distinct molecular mechanisms (Figure 6-4).[18] Granules present in the cytoplasm of activated CTLs contain molecules involved in causing cell lysis. These include perforin and granzymes. When the CTL contacts the target cell, the granules exocytose into the intermembrane space, where perforin (a complement-like molecule) causes the creation of pores in the target membrane. This initiates membrane destruction, which leads to an osmotic death associated with target cell necrosis. It also facilitates penetration into the target cell of the granzyme molecules released by the CTLs, leading to programmed cell death (apoptosis) of the target cell. In addition, some CTLs also express cell surface effector molecules that specifically interact with cellular death receptors. The best characterized of these is the Fas-Fas ligand system wherein activated CTLs express Fas ligand, which ligate the Fas receptor on target cells and induce apoptosis. Recently, other members of the Fas ligand family have been described. One of these, termed *TRAIL* for tumor necrosis factor (TNF)–related apoptosis-inducing ligand, is of particular interest in oncology because the TRAIL receptors (TR1 and TR2) appear to be restricted to neoplastic tissues. Recent work has shown that Ewing's sarcoma is particularly susceptible to death by TRAIL. [19]

**TABLE 6-1. CLUSTER DESIGNATIONS (CD) ASSIGNMENTS USED IN THIS CHAPTER**

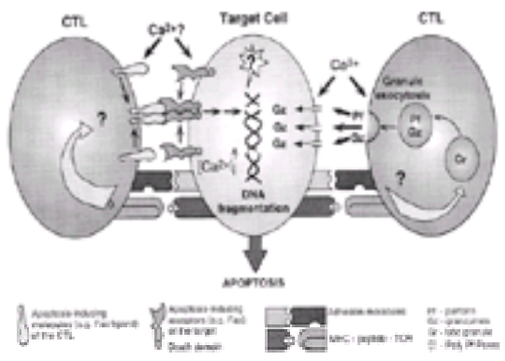


**FIGURE 6-4.** Mechanisms of lymphocyte-mediated cytolysis. Shown is a schematic diagram of the nonsecretory, receptor (e.g., Fas and TRAIL)-mediated (*left*) and the secretory, perforin/granzyme-mediated (*right*) mechanisms of lymphocytotoxicity. CTL, cytotoxic T cell; MHC, major histocompatibility complex; TCR, T-cell receptor. (Adapted from Berke G. Unlocking the secrets of CTL and NK cells. Immunol Today 1995;16:334.)

All T cells, including CTLs, can secrete soluble mediators (see section on cytokines). Nevertheless, a distinct population of T cells, designated helper T cells (Th), can be distinguished from CTLs, as Th cells express the CD4 surface molecule, instead of the CD8 molecule expressed on the surface of CTLs. When Th cells are activated, through recognition of antigen through their T-cell antigen receptors, they become metabolically activated, begin to proliferate, and secrete cytokines. Th cell populations can be further subdivided based on the cytokines they preferentially secrete and their distinct “helper” roles. Th1 helper T cells play a prominent role in helping the activation of CTLs and cellular immune reactions, and primarily secrete IL-2 and IFN-g. Th2 cells are involved in helping activation of B cells and can regulate (downmodulate) certain cellular responses. They secrete primarily IL-4, -5, and -10.

### *Targets of T-Cell Recognition: Role of the Major Histocompatibility Complex in Recognition by the T-Cell Receptor and the CD4/CD8 Cell Surface Receptors*

Elucidation of the molecular mechanisms responsible for rendering TCRs diverse enough to respond to the vast array of antigens, which they may encounter during the lifetime of the host, represented a seminal advance in the field of immunology. However, equally important were the observations made by cellular immunologists who gradually identified the targets of the recognition of these highly diverse abTCRs. Initially, it was erroneously assumed that, like antibodies, T cells recognized intact macromolecules present on the surface of the cells they recognize, designated *target cells* by immunologists. Since that time, a series of experimental observations by many investigators provided the critical insight that instead, the abTCRs of virtually all T cells recognize protein fragments (termed *peptides*) that are held by peptide-binding molecules (the MHC structures) that exist on the surface of virtually all cells. For $CD8^+$ cells, the peptides are generated intracellularly within the proteasome and are subsequently processed into small peptide units in the endoplasmic reticulum before their presentation on the cell surface by MHC class I molecules. For $CD4^+$ cells, the peptides are generated in the endosome of cells after ingestion of exogenous protein and then presented on the cell surface by MHC class II molecules.

This insight began in 1974 with the seminal observations by Zinkernagel and Doherty. They showed that T cells reactive to viral infection with lymphocyte choriomeningitis virus could only recognize virus in two different cells if MHC antigens were shared by the cells, even if the virus was identical in both systems. [20] This general property, wherein identical T cells can only recognize targets that share both the same viral infection as well as the same MHC molecule, was termed *MHC restriction* and was subsequently confirmed in a variety of other viral systems.[21,22] For some time, however, the mechanisms responsible for MHC restriction remained unclear. Initial explanations suggested that CTLs recognized whole proteins inserted alongside the membrane-bound MHC molecules. This proposed mechanism was found to be incorrect, as nuclear proteins that were not expressed on the cell surface could also be recognized by certain MHC-restricted T cells. [23,24] Indeed, in the extensively studied model of T-cell–mediated responses to influenza virus, cytoplasmic and nuclear proteins, rather than cell surface glycoproteins, were the preferred target antigens recognized by T cells. Furthermore, influenza-infected target cells recognized by antigen-specific CTLs showed no evidence for surface expression of the full influenza protein when assayed via binding by antiinfluenza monoclonal antibody. [25]

Subsequent studies confirmed that CTLs could specifically kill target cells that were not infected with influenza, but instead were first treated with exogenous administration of peptides 9-25 amino acids in length derived from whole influenza protein. This was followed by crystallographic analysis of the common HLA molecule, HLA-A2, which revealed a peptide-binding groove that could bind peptides of this size. [26] The HLA class I molecules (i.e., HLA-A, B, and C) and HLA class II molecules (i.e., the HLA-D region molecules, consisting of HLA-DP, -DQ, and -DR) have similar three-dimensional structures on the surface of the cell membrane. They consist of two a helices protruding parallel to one another and above a b-pleated sheet. This conforms to a large protein with a groove. To be visible to a reactive T cell, a peptide must fit exactly into the groove of an HLA molecule.

Progress in the understanding of the biochemistry and cell biology of antigen presentation has been on the forefront of immunologic advances in recent years (reviewed in ref. 27). Current paradigms hold that endogenous cellular proteins undergo proteolysis and processing in the proteasome ( Fig. 6-3). Peptide fragments are then transported into the endoplasmic reticulum via specialized molecules, which bind and transport peptides across the membrane of the endoplasmic reticulum via an energy-dependent process. These molecules have been termed *transporters of antigen processing*. The transporters of antigen processing molecules transfer their peptides to associated unstable MHC class I /$b_2$-microglobulin dimers. The binding of peptide to the MHC class I/$b_2$-microglobulin dimer gives rise to a stable trimolecular complex that is transported to the surface of the cell and is presented within the peptide-binding groove of MHC class I. Although the factors that determine the extent to which individual peptides bind to MHC molecules remain incompletely understood, it is clear that the extensive polymorphism that exists among MHC molecules results in substantial differences in the binding of individual peptides across individuals (reviewed in ref. 28).

Importantly, the MHC molecule also plays a critical role in directing peptides of appropriate affinity to $CD4^+$ versus $CD8^+$ cells. This is because the nonpolymorphic region of the MHC class I molecule binds to the CD8 molecule on T cells whereas the nonpolymorphic region of MHC class II binds to the CD4 molecule on T cells. In this way, $CD8^+$ T cells are brought into proximity of MHC class I expressing cells to recognize antigen whereas $CD4^+$ T cells only recognize antigen in the context of class II expression. Furthermore, class II molecules receive peptides in large part via the endosome that is generated as a result of pinocytosis and endocytosis of exogenous protein by class II expressing APC populations. In contrast, class I molecules receive peptide via transfer of peptides from the proteosome into the endoplasmic reticulum as noted. In this way, MHC class II primarily presents “exogenous” proteins to T cells, whereas MHC class I primarily presents endogenously generated peptides. Another critical difference between class I and class II MHC molecules is the limited distribution of MHC class II. Although MHC class I is expressed on essentially all nucleated cells, MHC class II expression is typically limited to professional APCs that have evolved specialized capacities to interact with the immune system (monocytes, dendritic cells, B cells, activated T cells, and endothelial cells).

The identification of the targets of T cell recognition were of considerable importance to the general immunology community because it provided a means to explain how the T-cell arm of the immune system evolved to eradicate viral pathogens that generate intracellular antigens as a result of target cell infection. [22] With regard to tumor immunology, the paradigm also suggested that a wide array of tumor-specific or tumor-associated proteins, even those restricted in distribution to the cytoplasm or nucleus, could potentially serve as targets for cell-mediated immune responses.

### *Critical Role of Costimulation: Antigen-Presenting Cells*

The model described paints a picture wherein enormous arrays of potentially recognizable self-peptides are continually presented on the surface of cells via binding to MHC molecules throughout the life of the host. To prevent autoimmunity, it is clear that mechanisms to control the reactivity of T cells bearing potentially autoreactive TCRs must exist. Experimental evidence accumulated in the 1990s has provided new insights into the processes that regulate T-cell activation. The primary concept that has evolved is that T-cell activation requires not only the interaction of an appropriate TCR with its cognate antigen (i.e., the first signal), but also requires a second “costimulatory” signal. This second signal is most frequently provided by direct interaction with costimulatory molecules present on the peptide-bearing cell. The best characterized, and the most potent, of the costimulatory signals involves the B7 family of molecules, which are expressed on the surface of professional APCs. B7-1 (CD80) and B7-2 (CD86) are surface glycoproteins that interact with CD28 on the surface of T cells. [29] Cross-linking of CD28 during T-cell activation enhances proliferation of responding T cells in part by upregulating IL-2 production. An important negative regulatory system also is incorporated into the CD28/B7-1/B7-2 interaction ( Fig. 6-5). Here, CTLA4, a molecule with very high affinity for both B7-1 and B7-2, competes with CD28 for binding

with these molecules. Rather than transmit activation signals to T cells as CD28 does, CTLA4 ligation results in downregulation of T-cell activation. [30] Under normal circumstances, this downregulation functions to limit the magnitude of the immune response, thus diminishing the likelihood of autoimmunity and lymphoproliferation. It has been shown in mouse models that if blockade of CTLA4 is induced during the course of an immune response, significant enhancement of antigen-specific responses occurs indirectly due to an enhanced interaction between CD28 and B7-1, B7-2. This approach has been used to induce primary responses to otherwise nonimmunogenic tumors in mouse models and could provide a therapeutic approach toward human cancer in the future. [31,32]

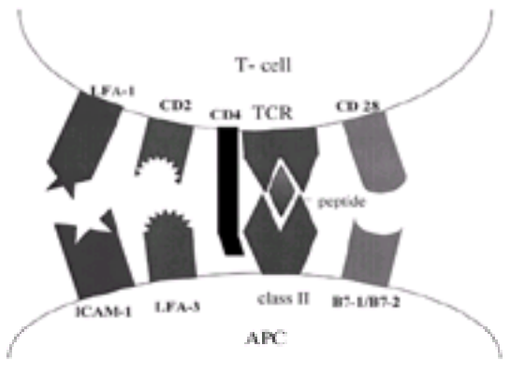


**FIGURE 6-5.** Costimulatory interactions between $CD4^+$ T cells and antigen-presenting cells (APCs). APCs express class-II HLA molecules, with antigenic peptides in their groove. These are recognized by the T-cell receptors (TCRs) on T cells. Additional interactions occur between the CD4 molecule on the T cell and the nonpolymorphic region of the class II HLA molecule. In addition, binding interactions occur between leukocyte factor antigen-1 (LFA-1), CD40 ligand, and CD28 molecules on T cells; and intercellular adhesion molecule-1 (ICAM-1), CD40 and CD80 (B7-1), and CD86 (/B7-2) molecules on APCs, respectively. CTLA4 acts as a negative regulator of T-cell activation and competes with CD28 for binding to CD80 and CD86 on APCs.

CD40 also appears to be a particularly important costimulatory molecule expressed on APCs. Ligation of CD40 via CD40 ligand expressed on activated $CD4^+$ T cells leads to upregulation of a multitude of other molecules on the APCs, including B7-1, B7-2, and MHC molecules. [33] Other accessory molecules present on the surface of professional APCs, such as intercellular adhesion molecule-1 and -2 and function associated antigen-3 (LFA-3) can also provide costimulatory requirements. [34] The two-signal model has also been described as the "danger" model wherein the expression of costimulatory molecules on APCs is induced via a variety of "danger" stimuli, including lipopolysaccharide and IL-12 production via cells of the innate immune system. In this model, the innate immune system provides a critical role by responding to a variety of "danger" signals by upregulating costimulatory molecules on appropriate APCs with subsequent activation of T cells bearing appropriate TCR specificities. [35]

This "two-signal" or "danger" model provides an explanation for why autoimmunity does not occur regularly despite the plethora of peptides expressed throughout the body in the context of MHC. With regard to tumor immunity, elucidation of the requirement for the second signal in immune response induction also provides an attractive explanation for the inability of tumors to induce immune responses in the host despite the existence of tumor-specific proteins, such as fusion proteins, which result from chromosomal rearrangement. This hypothesis generated the testable prediction that if tumor-associated antigens or tumor-specific proteins were expressed on the surface of professional APCs, which are capable of providing costimulatory requirements, then immune responses would be generated. Indeed, it has now been shown that in many experimental systems, effective antitumor immunity can be induced by enhancing costimulation of tumor-associated proteins. This has been accomplished via transfection of tumor cells themselves with various cytokines or costimulatory molecules, [36,37,38] and [39] or by immunization with professional APCs that have been manipulated to express tumor antigens. [40]

The same effect also occurs if large numbers of professional APCs are brought into proximity with tumor, as shown by Dranoff and co-workers. [38] Here, a process termed *cross-priming* results in pickup of tumor-associated peptides by infiltrating APCs, which are activated to respond to "danger." Indeed, it is possible that the phenomena of cross-priming is a critical process *in vivo* for inducing immune responses to dead and dying cells because recent work suggests that professional APCs acquire peptides from dead and dying cells via ingestion and subsequent presentation. [41,42]

The importance of costimulation for the induction of immune responses has fueled great interest in the study of cell populations capable of antigen presentation and costimulation. Of all APCs, dendritic cells are the most potent for inducing immune responses, [43] and they are unique among APC populations for their ability to induce responses from naive T cells to antigen. [44] Indeed, because a prevailing paradigm holds that the primary factor limiting antitumor responses is a lack of appropriate costimulatory molecules in the initial phases of the host-tumor interaction, much of the enthusiasm in tumor immunology today rests on the ability to isolate and activate large numbers of dendritic cells for use in the context of immunotherapy. Dendritic cells are normally found in trace amounts (1% to 2%) in blood and bone marrow, and are typically lacking in expression of T-cell, B-cell, and myeloid markers (e.g., CD3, CD14, CD16, and CD19). They express high levels of MHC class I and class II and generally express B7-1 and/or B7-2. Recent studies [45] have shown that large numbers of dendritic cells can be generated after incubation of peripheral blood monocytes with granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4. This leads to a nonproliferative differentiation of monocytes into cells with dendritic-like processes and modest upregulation of costimulatory molecules. These cells have been termed *immature dendritic cells*. They continue to possess the ability to ingest and process proteins (similar to monocytes), but they also show some increase in the ability to present antigen. After further stimuli (e.g., TNF-a, monocyte-conditioned media, lipopolysaccharide, and exposure to CD40 ligand) the immature dendritic cells undergo irreversible differentiation into cells with very high levels of costimulatory molecules. These cells have been termed *mature dendritic cells*. Substantial numbers of mature dendritic cells can also be generated *ex vivo* from $CD34^+$ hematopoietic pluripotential cells after culture with GM-CSF and TNF-a. This occurs via proliferation and differentiation of myeloid progenitors into a dendritic cell phenotype. Another approach for generating large numbers of dendritic cells involves *in vivo* treatment with flt3 ligand. Systemic treatment with this molecule also induces the differentiation of early myeloid progenitors into cells bearing a dendritic cell phenotype, thus resulting in the accumulation of large numbers of dendritic cells throughout the organs of flt3-treated hosts. [46] Properties of the various dendritic cell populations are shown in Table 6-2, and important costimulatory interactions between APCs and T cells are shown in Figure 6-5. Ongoing work is attempting to clarify the optimal dendritic cells for induction of T-cell–mediated immune responses for ultimate use in antitumor immunotherapy.

| | Monocyte | Immature dendritic cell | Mature dendritic cell |
|---|---|---|---|
| Cell surface markers | $CD14^+$<br>HLA-DR lo<br>$FcR^+$<br>CD1a lo<br>CD80 lo, CD86 lo<br>CD83 lo | $CD14^-$<br>HLA-DR mod<br>$FcR^+$<br>CD1a hi<br>CD80 mod, CD86 mod<br>CD83 lo | $CD14^-$<br>HLA-DR hi<br>$FcR^-$<br>CD1a lo<br>CD80 hi, CD86 hi<br>CD83 hi |
| Antigen uptake | ++ (phagocytosis) | ++ (phagocytosis and macropinocytosis) | – |
| Antigen processing | + | ++ | – |
| Antigen presentation | – | ++ | ++++ |

**TABLE 6-2. PROPERTIES OF ANTIGEN-PRESENTING CELL POPULATIONS**

## B Lymphocytes

B lymphocytes (designated *B* from their bursal origin in fowl and bone marrow origin in mammalian species) are characterized when mature by having a surface membrane receptor composed of the antigen-binding component of an Ig molecule. The primary role of B lymphocytes is the production of intact Ig molecules, which are soluble molecules able to recognize protein, glycoprotein, and carbohydrate antigens and trigger other cellular and molecular responses on effective binding of

antigens they recognize.[47] As such, B lymphocytes are primarily designed to recognize and respond to soluble intact antigens, and the Igs they produce bind to epitopes that are available on the surface of whole circulating molecules. This is to be distinguished from T cells, which do not recognize intact proteins, but rather peptides that must be presented on the cell surface of MHC-bearing cells. Each Ig molecule consists of light (k or l) chains and heavy (M, G, A, D, or E) chains. Intact Ig molecules have an antigen-binding (Fab) end and an end that can fix and activate components of the complement cascade (Fc) and can bind to membrane-bound Fc receptors when the Fab end of the antibody is engaged with the appropriate antigen.

Like the genetic control of T-cell diversity, germline and nonimmune cells all contain the same pattern of germline Ig genes. As immune stem cells begin differentiating into B-lymphocyte lineages, Ig gene rearrangements involving selection from multiple segment possibilities within V, D, and J regions are rearranged. [48] When these possibilities are coupled to the different major types and subtypes of heavy and light chain constant regions that can be selected, the genetic control of an individual's immune repertoire may allow $10^{11}$ different combinations of Ig (Table 6-3). At any time, a single B cell and its progeny can secrete many copies of a single specific Ig molecule. Different B cells, having different Ig gene rearrangement patterns, make distinct Ig molecules. Efficient activation of B cells to secrete Ig molecules requires interactions with antigen-specific T cells that have also become activated during the course of immune response induction. Although some of this T cell “help” is accomplished via secretion of cytokines, such as IL-2, it is now clear that much of the help provided by T cells is in the form of the cell-associated molecule, CD40 ligand. This ligand, expressed on the surface of activated CD4 T cells, can activate CD40 on the surface of B cells to direct them to generate antigen-specific IgG-producing cells. Indeed, humans lacking CD40 have a defect in isotype switch resulting in an accumulation of IgM molecules, known as the *hyper IgM syndrome*.

| | Immunoglobulin | | αβTCR | | γδTCR | |
|---|---|---|---|---|---|---|
| | H | L | α | β | γ | δ |
| Variable segments | 250–1,000 | 250 | 100 | 25 | 7 | 10 |
| Diversity segments | 10 | 0 | 0 | 2 | 0 | 2 |
| Joining segments | 4 | 4 | 50 | 12 | 2 | 2 |
| Variable region combinations | 62,500–250,000 | | 2,500 | | 70 | |
| Junctional combinations | $10^{11}$ | | $10^{11}$ | | $10^{11}$ | |

Note: Estimated amino acid sequence diversity in immunoglobulin and TCR genes without allowance for somatic mutation. Estimates for the number of amino acid sequences that might result from diversity within the junctional region are contrasted for immunoglobulin and TCRs. Mechanisms for diversity generation within the junctional region include usage of different diversity (D) and junctional (J) gene segments, junctional N region additions, variability in the joining position of variable and J gene segments, and translation of D regions in different reading frames.
Adapted from Davis MM, Bjorkman P. The T-cell antigen receptor genes and T-cell recognition. Nature 1988;334:395.

**TABLE 6-3. DIVERSITY IN T-CELL RECEPTOR (TCR) AND IMMUNOGLOBULIN GENES**

Just as molecular characterization of TCR gene patterns can differentiate polyclonal from oligoclonal or monoclonal T-cell populations, the same is possible for Ig gene rearrangement patterns among B-cell populations. This information has been of use in characterizing a variety of B-cell tumors. Because mature B cells express Ig on the cell surface corresponding to the Fab type of the Ig they are genetically programmed to secrete, monoclonal B-cell tumors consist of a population that all express the identical surface Ig Fab type. That pattern on a monoclonal population of malignant B cells [49] allows those cells to be distinguished from normal B cells not expressing the same Ig surface receptor (i.e., the Ig idiotype). This distinction has enabled idiotypes to function as tumor-specific markers, and these idiotypes have been the target for immunologic therapy.[50] Here the peptidic immune target for the T cell is the idiotype portion of the antibody itself; T-cell responses are generated that attack any cell bearing this idiotype.

Of tremendous importance to diagnostic and clinical immunology was the hybridoma technique that used murine B-cell tumors to make large quantities of monoclonal antibodies.[51,52] A hybridoma is formed by fusing a malignant B cell (Fig. 6-6) that is a poor Ig secretor with a normal B cell that makes an Ig of interest. [53] With current genetic engineering techniques, molecularly modified antibodies are being created with a number of laboratory designer improvements. Genes controlling the antigen-binding component of an Ig molecule can be fused to genes controlling constant regions for other Ig molecules. Chimeric antibodies are being created that allow mix and match combinations of antigen binding and constant regions using genes from different cells or even different species to provide the desired antigen recognition and biologic function.[54] The technology is moving toward *in vitro* gene recombination and cyclical, sequential mutation and recombination to generate Igs that may not be readily found in immune cells. The generation[55] of such *in vitro* generated and modified molecules can provide specificities and functions not found in nature, and these may have multiple clinical uses.

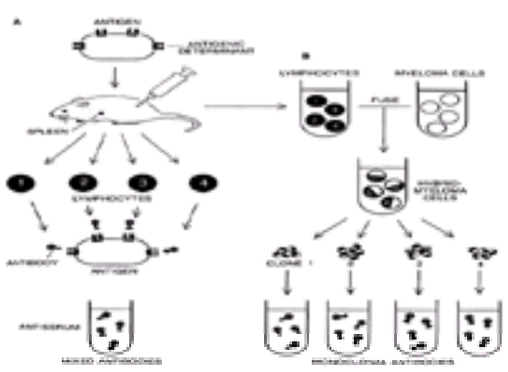


**FIGURE 6-6.** Generation of monoclonal antibodies. Immune response is initiated **(A)** when an antigen molecule carrying several different antigenic determinants enters the body of an animal. The immune system responds, and lines of B lymphocytes proliferate, each secreting an immunoglobulin molecule that fits a single antigenic determinant or a part of it. A conventional antiserum contains a mixture of these antibodies. **B:** Monoclonal antibodies are derived by fusing lymphocytes from the spleen with malignant myeloma cells. Individual hybrid cells are cloned, and each of the clones secretes a monoclonal antibody that fits a single antigenic determinant on the antibody molecule. (From Milstein C. Monoclonal antibodies. Sci Am 1980;243:66, with permission. Reprinted in Paul WE. Immunology: recognition and response. New York: WH Freeman, 1991:125.)

## Natural Killer Cells

Morphologic evaluation of peripheral blood leukocytes enables easy separation of mononuclear cells from polymorphonuclear leukocytes and relatively clear distinction of lymphocytes from monocytes within the mononuclear cell fraction. Within the population of peripheral blood mononuclear cells that appear to be lymphocytes by standard hematologic smear analysis and by standard flow cytometric techniques, there is a relatively small population of circulating cells that does not express mature T- or B-cell markers (CD3 or Ig). This third population of lymphocytes ( Fig. 6-2), initially designated *null cells*, has now been found to be comprised predominantly of a cell type termed *NK cells*. NK cells are notable for their ability to spontaneously destroy some tumor cells lines and virally infected populations *in vitro*.[56]This cytolytic capacity is mediated via the same molecules used by cytolytic T cells (i.e., perforin, granzyme, and Fas-Fas ligand interaction), as shown in Fig. 6-4, in inducing target cell lysis. Unlike T cells, however, which require preactivation to induce expression of their perforin and granzyme containing granules, NK cells constitutively express cytolytic granules and thus can kill appropriate targets very rapidly. Indeed, the observation of such granules histologically has historically led to the phenotypic description of NK cells as large granular lymphocytes.

The molecules responsible for signaling NK cells to recognize and then kill their targets have been difficult to characterize. It is clear that NK cells do not express the TCRs or Ig molecules that are present on T and B cells, respectively. [57] Furthermore, experimental evidence has shown that rather than requiring MHC expression on target cells for induction of cytolysis (as is the case for T cells), NK cell lysis is actually inhibited by the presence of MHC molecules on the targets they recognize. This has given rise to the current model for NK cell recognition, termed the *missing self hypothesis*.[58] In this model, under normal circumstances NK cells are prevented from granule release by ongoing “tonic” engagement of inhibitory receptors on the surface of NK cells that occurs via their interaction with self-MHC. Indeed, substantial progress in the identification of such inhibitory receptors has been made in the last several years. NK inhibitory receptors generally belong to one of at least three major families: first, the killer inhibitory receptors (KIRs), which are most well characterized in humans and that are comprised of an Ig-like molecule;

second, the CD94/NKG2 family of lectin type molecules that is predominantly characterized in humans; and finally, the Ly49 family, which is seen in mice and is comprised of type II transmembrane protein dimers (reviewed in ref. 59). Importantly, however, there appears to be great diversity within these systems of NK receptors, with a large diversity of KIRs expressed on individual NK cells and specificity for individual KIRs for individual MHC alleles. Furthermore, in some cases, molecules contained within these families of inhibitory receptors can also activate NK cells.

The current “missing self” model explains why NK cells may preferentially attack neoplastic cells that are known to downmodulate MHC molecules. In theory, such a model suggests that NK cells would provide an “immune surveillance” mechanism selectively targeting tumor cells or virally infected cells that downmodulate MHC molecules. Concurrently, the T-cell arm of the immune system would be poised to lyse MHC-bearing cells, which express foreign antigens. Importantly, however, recent work has shown that certain subpopulations of T-cell populations show many features of NK cell reactivity and also express KIRs, suggesting the potential for downmodulation of such NK/T-cell populations via MHC-bearing cells. With regard to tumor immunology, one could envision a scenario wherein downmodulation of one MHC allele that may be responsible for presenting a particular tumor-specific peptide may diminish the tumor's capacity to be recognized by classical T cells, whereas maintenance of another allele, reactive with the KIR receptors, could turn off NK-cell–mediated cytolytic responses. In this way, the tumor could subvert both the T-cell and NK-cell immune response.

The physiologic roles for NK cells are likely to be manifold. [56] First, these cells appear to contribute to a first line of defense against viral pathogens because hosts that are deficient in NK function show a great susceptibility to infection with herpes viruses. In addition, NK cells serve a unique position as communicators between innate and adaptive immune responses. To this end, NK cells are highly responsive to IL-12 production by monocytes and other tissue macrophages and respond with the production of high levels of IFN-g. Such IFN-g expression appears to play an important role in directing Th cell responses toward a Th1 phenotype. The Th1 phenotype has been proposed to be responsible for the induction of cell-mediated immunity and in some models is required for protection against a variety of infections. Furthermore, because of their capability for tumor lysis, it has long been postulated that NK cells contribute to a system of immune surveillance wherein the accumulation of cells lacking self-MHC molecules (such as tumor cells) would be eradicated. Indeed, in animal models, it is clear that abrogation of NK-mediated killing greatly facilitates the ability of tumor cell lines to grow in immunodeficient mice.

Importantly, NK cells also express the CD16 molecule, which is the low-affinity FcRIII that can bind to the Fc end of Ig molecules, provided the antibody is in the process of recognizing the appropriate antigen with its Fab end. Through this receptor, NK cells can potently mediate destruction of other cells that are coated with antibody via antibody-dependent cellular cytotoxicity (ADCC). This process is predicted to be of central importance for the efficacy of tumor-directed monoclonal-based therapy, and recent approaches integrating IL-2 into chimeric monoclonal antibodies have attempted to enhance ADCC by increasing the binding of monoclonal antibodies to NK cells via the IL-2 receptor. [60]

Finally, NK cells also show striking responses to IL-2, a cytokine released by Th cells ( Table 6-1). *In vitro* and *in vivo*, exposure to IL-2 causes activation and proliferation of NK cells, producing changes readily detectable by molecular and morphologic techniques. [61,62] Functionally, these IL-2–activated NK cells mediate *in vitro* destruction against an even broader range of tumor cell lines (compared to that killed by fresh NK cells). [61] Some lesser degrees of killing are observed by these IL-2–activated NK cells on certain fresh normal tissues, including monocytes and endothelial cells. [63] These IL-2–activated NK cells are largely responsible for the beneficial effects of lymphokine-activated killer (LAK) cell therapy seen in some patients with renal cell carcinoma and melanoma (see Chapter 14). [64,65]

## Cytokines

### *Intercellular Immunoregulation by Soluble Signals*

The complete *in vivo* immune response involves a tightly orchestrated interaction of all of the previously described cell types, requiring a coordinated activation or inhibition of each component of the response at the correct place and time. Many of these interactions involve direct cell contact between different cell types or different cells of similar types. However, most interactions occur through the use of soluble mediators, designated *cytokines* or *lymphokines* when produced by lymphoid cells. Because these molecules provide communication between different leukocytes, some have been given the designation of *IL*. [66] More than 18 different ILs have been characterized (Table 6-1). The family of IFNs and hematopoietic growth factors reflect protein molecules that are made by cells of the immuno-hematopoietic system and also have direct regulatory effects on the immuno-hematopoietic response. [67,68] A separate class of cytokines are those that are involved in the attraction of leukocytes to areas of inflammation. As this “chemical mediated” process of cellular attraction has been designated *chemotaxis*, the cytokines involved in this process are known as *chemokines*. [69] Synthesis of each of these molecules is regulated by cellular activation, and these molecules are specifically recognized by cell membrane receptors that trigger cellular differentiation and nuclear activity by receptor-bearing cells. The molecular pathways involved in these activation steps are somewhat analogous to that of T-cell triggering through the CD3 triggering molecule.

### *Interleukin-2 and Its Receptor*

Initially described as T-cell growth factor, IL-2 is a 15-kd protein that has been studied in greater depth than any of the other ILs. It is produced by Th1 cells after specific antigen recognition and produces differentiation and proliferation of other immune cells, specifically Th cells, CTLs, and NK cells, as well as less striking effects on B cells and monocytes. [69,70] IL-2 is able to activate cells of the immune system if they bear a functional IL-2 receptor. At a minimum, the IL-2 receptor consists of three distinct membrane receptor components, the a, b, and g chains. The intermediate-affinity IL-2 receptor consists of the 75-kd b chain that has extracellular, transmembrane, and cytoplasmic domains, as well as a 65-kd g chain that also has extracellular, transmembrane, and cytoplasmic components. The high-affinity receptor is composed of one b chain, one a chain, and one g chain, which is a 55-kd molecule with only a minute cytoplasmic domain. [68,69 and 70] Most T cells and NK cells express low amounts of the b and g chains and virtually no a chain. After recognition of specific antigen, T cells are activated to synthesize the a chain, enabling formation of a heterotrimer consisting of an a, b, and g chain that provides for high-affinity IL-2 receptor signal transmission ( Fig. 6-7). Activated T cells express the a/b/g trimolecular complex. This expression has been used to identify T cells that have recently recognized antigen, and immunosuppressive approaches directed against cells expressing this IL-2 receptor (IL-2R) complex are being used to treat autoimmune disease and allograft rejection episodes. [69] Similar approaches are being initiated for treatment of T-cell malignancies.

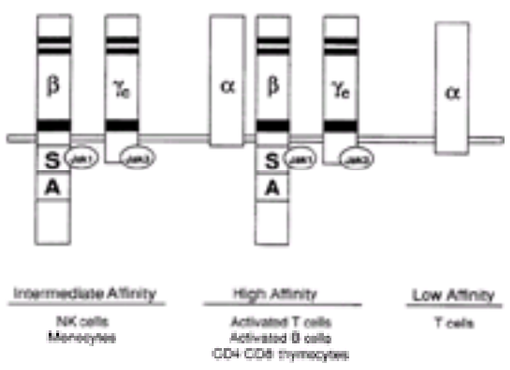


**FIGURE 6-7.** Interleukin-2 receptor (IL-2R) forms and the immune cells that express them. Individual subunits that compose the three forms of IL-2Rs are as indicated. JAK1 is a JAK family kinase that associates with the S (serine-rich) region on IL-2Rb and is a primary kinase associated with IL-2 signaling. JAK3 is a JAK family kinase that associates with IL-2Rg and is required for IL-2 signaling. The acidic (A) region is also required for proper signaling to occur. Immune cell subsets that express each IL-2R form are listed below the receptor schematics. (From Farner NL, Hank JA, Sondel PM. Molecular and clinical aspects of interleukin 2. In: Friedland JS, Remick DG, eds. Cytokines in health and disease, 2nd ed. New York: Marcel Dekker, 1997;29–40, with permission.)

In addition to expressing the a/b/g IL-2R complex on the cell surface, activated T cells, while synthesizing the a IL-2R molecule, also secrete this molecule in large quantity. Its release into the serum can be quantified and correlates with the degree of immunologic activation under way. NK cells also respond to IL-2 using what appear to be the same IL-2R molecules. “Resting” NK cells appear to have more of the 75-kd b chain than do resting T cells. [71] Although some NK cells can activate a IL-2R expression after immune activation (particularly activation with IL-2), others appear to increase their b chain expression but do not express IL-2R a chains. Functional studies with these NK cells document that the g chain plays a critical role in IL-2 binding and signal transduction. This same g chain receptor molecule is

also essential as a receptor involved in the response to a number of other interleukins (e.g., IL-2, -4, -7, -9, -13, and -15). [52,72] This is evident from the critical role this common g chain receptor molecule plays in immune development. Individuals unable to produce a normal functioning common g chain are afflicted with X-linked severe combined immunodeficiency, most likely related to a deficiency in IL-7 signaling. [71]

### *Other Cytokines*

Although IL-2 has been studied most extensively, the diverse array of other cytokines that have been identified indicates the complexity of intercellular communication through soluble mediators used by the immune system. Some of these are described briefly in Table 6-4. A detailed description of the known functions, physiologic roles, and potential immunotherapeutic implications for each is beyond the scope of this chapter. [73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89] and [90]

**TABLE 6-4. HUMAN CYTOKINES**

## IMMUNODEFICIENCY IN CANCER PATIENTS

### Immunodeficiency and Cancer Predisposition

Since the mid-1980s, experimental oncology has been enriched by techniques allowing highly sophisticated analyses of malignant cells and the genes within them. Numerous investigations have demonstrated that malignant differentiation is a multistep process involving expression of abnormal genes heralded by characteristic structural changes in the proteins and other molecules expressed by many malignant cell populations. Delineation of this complex multistep process of neoplastic differentiation has been used to argue against the immune surveillance hypothesis, which holds that, in health, numerous neoplastic or pre-neoplastic cells arise spontaneously and are destroyed by an effective immune system before progressing to cancer. The association of increased incidence of malignancy in patients (particularly children) with severe immunodeficiency disorders was cited to support the immune surveillance concept.

Despite the currently controversial status of the immune surveillance hypothesis, the clinical facts remain: Patients with severe immunodeficiency disorders show dramatic increases in the incidence of certain neoplasms. Because many of these immunodeficiency diseases are extremely rare, the identification of a neoplasm in a pediatric patient suggested an increased risk of cancer was associated with immunodeficiency, but it was based largely on the anecdotal observation of two rare diseases in the same patient. Establishment of the Immunodeficiency Cancer Registry has provided important data regarding the incidence of these immunodeficiency disorders and of neoplasms that arise in patients with these rare diseases. [91] Within the pediatric population, the severe immunodeficiency disorders associated with increased risk of malignancy include inherited forms of immunodeficiency such as severe combined immunodeficiency, [91] Wiskott-Aldrich Syndrome, [91] X-linked lymphoproliferative syndrome, [92] ataxia telangiectasia, and selective IgA deficiency. Increased incidence of malignancy is also observed for patients with acquired forms of immunodeficiency such as patients with organ allografts, HIV and acquired immunodeficiency syndrome, prior treatment with cytotoxic drugs for a primary malignancy, and certain chronic viral infections. [93]

If loss of immune surveillance was the entire explanation for the increased incidence of cancer in these populations, the spectrum of neoplasms developing in immunodeficient pediatric patients should correspond to the spectrum developing in an age-matched pediatric population. Despite the increased incidence of neoplasms in these immunodeficient children, [91,94] the fraction that are lymphoid malignancies (particularly non-Hodgkin's lymphomas) is strikingly greater than the incidence of these diseases in the general population of pediatric oncology patients, whereas the fraction that are primary central nervous system malignancies is less than that of the general pediatric oncology population (see Chapter 1 and Chapter 2). However, the statistical association of immunodeficiency and neoplastic disease remains clear. Rather than proving or disproving the immune surveillance hypothesis, these clinical associations of immunodeficiency and cancer susceptibility suggest that several cellular and molecular mechanisms may be simultaneously at work.

First, molecular defects associated with genetic damage may simultaneously cause immune dysfunction and independently cause DNA damage associated with an increased susceptibility to neoplastic transformation. For instance, the neoplasms and immunodeficiency associated with diseases such as ataxia telangiectasia may have a common molecular cause, without a direct correlation between the immune dysfunction and neoplastic disease. Second, dysfunction of any physiologic system is often associated with a compensatory attempt to stimulate the missing function. Patients undergoing nephrectomy show hypertrophy of the remaining kidney. Patients with bone marrow hematopoietic stem cell dysfunction often show excessive levels of erythropoietin. Patients with end-organ failure of their T- and B-cell immune systems probably are experiencing some degree of lymphocyte and stem cell overdrive, attempting to ineffectively induce repopulation of the immune system. Indeed, recent evidence for increases in serum levels of IL-7 in association with chronic T cell depletion could provide a plausible mechanisms for the increased incidence of B-cell neoplasms because mice transgenic for IL-7 show a high rate of lymphoma development. [95] Third, a dysfunctional immune system may also be ineffective at providing immune control of infectious agents that are associated with or stimulate neoplastic differentiation (e.g., Epstein-Barr virus as the central factor in inducing posttransplant lymphoproliferative disorder or lymphoma, or both). [96]

Finally, it still remains a possibility that immune surveillance may play a part in preventing the development of cancer, even within the context of a multistep neoplastic differentiative process. Indeed, recent work in murine models has shown that mice that have been genetically engineered to have a predisposition to cancer (via disruption of the p53 gene) as well as to be deficient in IFN-g develop cancers earlier than IFN-g–producing mice. These results suggest that IFN-g, which is clearly produced by the innate immune system and a central player within the effector arms of the adaptive immune system, could contribute to immune surveillance in these mice. Similarly, mice treated with mutagenic agents develop tumors earlier when IFN-g is absent compared to mice with normal IFN-g production. [97] These results suggest a role for a least some component of immune surveillance in controlling tumors that arise as a result of genetic aberrations.

### Immunologic Effects of Cancer and Cancer Therapy

The most common infectious complications observed in cancer patients are bacterial infections occurring in the setting of neutropenia. [98] Bacterial infections are also commonly observed as a result of the breakdown of barriers (e.g., mucosal surfaces, indwelling central catheters, instrumentation, and surgery). In these settings, alterations in the innate immune system play a central role, with depletion of neutrophils being the most significant predisposing risk factor. Although acute depletion of neutrophils is clearly associated with a very high risk of infection, rapid recovery of these populations is generally observed. Indeed, as noted in Figure 6-8, neutrophils and monocytes recover rapidly after successive cycles of dose intensive chemotherapy, despite deep nadirs. Mechanistically, we know that hematopoietic cells (i.e., red blood cells, neutrophils, monocytes, and platelets) are short-lived, post-mitotic, terminally differentiated cells, which are continuously replenished throughout life via repetitive cycles of hematopoietic stem cell differentiation. As a result, when these cells are acutely depleted by cytotoxic antineoplastic therapy, complete repopulation generally occurs within 14 to 21 days via hematopoietic stem cell differentiation.

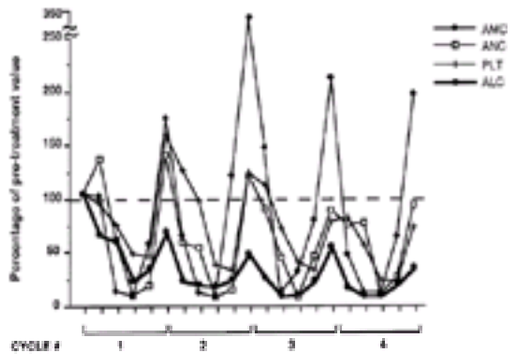


**FIGURE 6-8.** Peripheral blood lymphocytes show limited recovery after successive cycles of intensive chemotherapy. Temporal recovery of hematopoietic populations after successive cycles of dose-intensive chemotherapy as administered for pediatric sarcomas. Cycles were guided by recovery of neutrophils, which show baseline values before initiation of successive cycles. The graph displays an overshoot of the monocyte compartment. Platelet recovery is modestly reduced with successive cycles. The most limited recovery is seen in the absolute lymphocyte count (ALC), which does not return baseline at any time during successive cycles of chemotherapy. AMC, absolute monocyte count; ANC, absolute neutrophil count; PLT, platelet count. (From Mackall CL, Fleischer TA, Brown MA, et al. Lymphocyte depletion during treatment with intensive chemotherapy for cancer. Blood 1994;84:2221–2228, with permission.)

In contrast, T cells are comprised of a heterogeneous group of short- and long-lived cells, which are themselves capable of substantial mitotic expansion. Under normal circumstances, the long-lived cells, typically contained within the “naïve” subset, are quiescent, remaining in a noncycling state for months to years while awaiting encounter with cognate antigen.[99,100] The short-lived cells, generally contained within the effector and memory subsets, undergo variable levels of cell cycling in response to antigen encounter, resulting in ongoing modulation of their relative contribution to the overall T-cell repertoire.[101] Hence, in healthy hosts, ongoing hematopoietic stem cell differentiation plays only a minor role in maintaining peripheral T-cell populations. Furthermore, unique microenvironmental conditions present within the thymus are required for efficient T-cell differentiation from hematopoietic stem cells. Because age-, therapy-, and disease-related changes diminish the capacity of the thymus to produce new T cells, stem cell differentiation pathways for T-cell regeneration are frequently limited after T-cell depletion. It is therefore not surprising, that the acute depletion of T cells induced by cytotoxic antineoplastic therapy is often followed by a period of slow and incomplete restoration of peripheral T-cell populations.

For these reasons, cancer patients are also predisposed to a variety of nonbacterial infections with viral, fungal, and parasitic pathogens during the course of therapy and for some time after completion of antineoplastic therapy. Unraveling the precise contribution of specific alterations in innate versus adaptive immunity to specific infections is difficult. Nonetheless, it is clear that a deficiency in T-cell immune competence contributes to a susceptibility to viral infection as well as the development of other complications such as transfusion-associated GVHD and even Epstein-Barr virus–related lymphoproliferative disorder after allogeneic BMT.

Interactions between cancer and the immune system are complex. It is widely accepted that cancer patients display varying degrees of generalized immunosuppression at the time of clinical presentation before initiation of antineoplastic therapy. This appears to be most pronounced in patients with acute leukemia and other conditions associated with pancytopenia who frequently have infections at the time of presentation.[102] In this population, the susceptibility to infection is typically exacerbated by prolonged and intensive chemotherapy administered with or without corticosteroids.[103]

Similarly, patients with newly diagnosed Hodgkin's disease also frequently show impaired lymphocyte proliferation to a variety of antigens,[104] and patients with Burkitt's lymphoma have been reported to show variable levels of lymphocyte depletion that relate to the stage of disease.[105] Even patients with sarcoma sometimes show reduced peripheral blood T-cell populations at the time of presentation.[106] Despite these abnormalities in T-cell populations that are seen in cancer patients at the time of initial presentation, clinically profound T-cell immunosuppression is relatively uncommon before initiation of cytotoxic antineoplastic therapy.

After initiation of cytotoxic antineoplastic therapy, however, complications related to T-cell immunodeficiency are observed in a variety of clinical settings. Clearly, this is most severe and most frequent after allogeneic bone marrow transplantation when a variety of factors contribute to a high incidence of opportunistic complications (reviewed in ref. 107). Among these is the underlying disease, as patients with hematologic neoplasms who are referred for BMT have typically undergone intensive and prolonged therapy. Second, preparative regimens for allogeneic BMT are designed to be immunoablative. Indeed, it is clear that a primary factor necessary to allow engraftment of allogeneic hematopoietic stem cells is sufficient immunoablation to avoid host-versus-graft rejection. For this reason, common preparative regimens are highly immunosuppressive and frequently include a combination of cyclophosphamide and total body irradiation. More recently, the use of fludarabine has been shown to add enough to immunosuppression to allow engraftment without true myeloablation. Because of the profound lymphocyte depletion induced by fludarabine, it is anticipated that the risk for opportunistic complications will remain quite high in patients who receive this agent. T-cell depletion of the marrow graft also plays a significant role in enhancing immunosuppression by eliminating one potential pathway for T-cell regeneration ( Fig. 6-9). Finally, GVHD plays a pivotal role in adding to the immunosuppression associated with BMT. Not only does GVHD impact significantly on immune reconstitution, but the medications used to treat GVHD are potently immunosuppressive. Indeed, the most common cause of death in patients with GVHD is related to infectious complications. Therefore, recipients of allogeneic BMT are in general the most highly immunosuppressed group of cancer patients. For this reason, systematic approaches to prevent and treat opportunistic infection are indicated in the clinical management of these patients as discussed in Chapter 16 and Chapter 41.

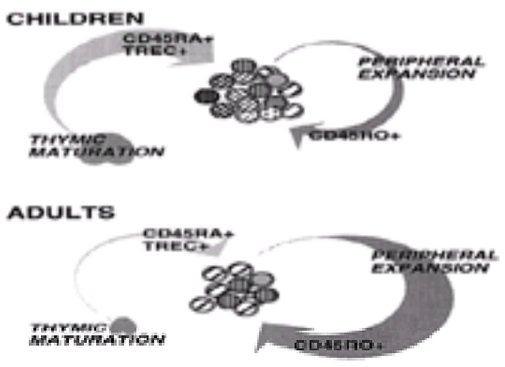


**FIGURE 6-9.** Pathways of T-cell regeneration after cytotoxic chemotherapy. Shown is a schematic representation of the two primary routes for $CD4^+$ T-cell regeneration. Children primarily regenerate T cells via thymic-dependent pathways as evidenced by normalization of peripheral $CD4^+$ T-cell numbers, a diverse T-cell receptor (TCR) repertoire, and the presence of rising numbers of $CD45RA^+/CD62L^+$ cells in the peripheral blood. In contrast, adults primarily regenerate $CD4^+$ T cells via the peripheral expansion of residual cells. This results in chronically reduced $CD4^+$ T-cell numbers, the accumulation of activated $CD4^+$ T cells ($CD45RA^+$, $HLA\text{-}DR^+$), a skewed TCR repertoire, and a propensity for programmed cell death. (Adapted from Mackall CL, Hakim FT, Gress RE. T-cell regeneration: all repertoires are not created equal. Immunol Today 1997;18:245–251.)

Notably, immunosuppressive complications of autologous BMT are generally less severe despite generally similar levels of initial T-cell depletion,[108,109] emphasizing the important role that GVHD and immunosuppressive medications used to control GVHD play in inducing allogeneic transplant-related immunosuppression. More recently, as the dose intensity of antineoplastic regimens has increased, opportunistic infections have also emerged as complications of cytotoxic antineoplastic therapy outside of the realm of bone marrow transplantation.[106,110,111] The agent most commonly implicated in antineoplastic therapy–induced immunosuppression is cyclophosphamide, which is capable of profound immunosuppression when administered as a single agent at high dose intensity.[112] In one study conducted at the National Cancer Institute (NCI) investigating single-agent dose escalation of cyclophosphamide, repetitive cycles with doses of 3.6 to 4.5 g per $m^2$ were administered in combination with GM-CSF. Sequential cycles were administered as rapidly as possible after myeloid recovery, which was unusually rapid, often within 14 days. In this study, neutropenic bacterial infections were uncommon, but opportunistic infections emerged as the dose-limiting toxicity. Therefore, although myeloid growth

factors allowed compression of the cycle length by minimizing myelotoxicity, the paradoxical result was an increase in regimen-related immunodeficiency. Similarly, recent results have shown that the use of autologous peripheral blood progenitor cell infusions can ameliorate myelosuppression after high-dose or myeloablative therapy; however, such infusions (at least in the autologous setting) do not lead to more rapid recovery of T-cell populations. [113] These results emphasize the distinctions in the regenerative pathways for myeloid versus T-cell populations after cytotoxic antineoplastic therapy. Although myeloid cells typically recover within 21 days after even dose-intensive therapy, lymphoid populations are reduced for a much longer period ( Fig. 6-8).

Clinically apparent immunosuppression has also been reported in patients enrolled on multiagent dose-intensive regimens administered for solid tumors and lymphoma. In one study, the dose intensity of the treatment regimen was shown to have a significant influence on the incidence of *Pneumocystis carinii* pneumonia, independent of other factors such as disease histology and stage at presentation. [114] Quantification of lymphocyte populations in the peripheral blood of patients treated on such dose-intensive protocols reveals significant T-cell depletion with a more profound effect on $CD4^+$ versus $CD8^+$ T-cell populations, resulting in a significantly reduced CD4:CD8 ratio. Although the relationship between the degree of $CD4^+$ depletion and the incidence of opportunistic infections has not been as extensively studied in cancer as it has in HIV infection, [115] there appears to be a general correlation between the degree of CD4 depletion and the risk for opportunistic infection in cancer patients as well. In HIV infection, it has been shown by many investigators that the risk for opportunistic infection rises substantially when the CD4 count falls below 200 cells per $mm^3$. Based on these data, the relationship between CD4 counts and opportunistic infection was compared among patients treated on three NCI protocols that showed variable levels of CD4 depletion. The rate of opportunistic infections was 0% with a mean posttherapy $CD4^+$ count of 120 ± 38 per $mm^3$, 33% when the mean posttherapy CD4 count was 68 ± 16 cells per $mm^3$, and 67% when the mean posttherapy CD4 count was 9 cells per $mm^3$.[106] Although the numbers of patients studied in this series was too small to definitely identify a threshold level of CD4 depletion that results in clinically significant immunosuppression, these results clearly show that chemotherapy as administered in the context of dose-intensive protocols for solid tumors in children can induce severe CD4 lymphopenia with a heightened susceptibility to opportunistic infection. This risk seems greatest when CD4 counts fall below 100 cells per $mm^3$.

As noted, the purine nucleoside analogs (2'-deoxycoformycin, 2-chloro-2'-deoxyadenosine and fludarabine monophosphate) represent another class of agents with a predilection for lymphocyte depletion. [116],[117],[118] and [119] These agents have a remarkable capacity for depletion of both dividing and resting lymphocytes, which likely contributes to their utility in the treatment of hairy cell leukemia and indolent lymphomas. Not surprisingly, however, these agents also induce profound depletion of normal lymphocyte populations, resulting in clinical complications related to opportunistic pathogens. As discussed, these agents are currently under investigation as the central components of new immunosuppressive preparative regimens used to allow hematopoietic stem cell engraftment in the setting of nonmyeloablative BMT.

Another clinical scenario wherein a substantial incidence of opportunistic complications occurs in the context of antineoplastic therapy involves chemotherapy regimens administered to patients with brain tumors.[120],[121] In this case, the combination of the T-cell depleting effects of chemotherapy and the functional alterations in cell-mediated immunity due to systemic corticosteroids results in significant immunosuppression. In addition, although radiation therapy in and of itself has modest immunosuppressive effects, one report suggests that the combination of radiotherapy and paclitaxel results in severe lymphocyte depletion when administered concurrently.[122] One regimen, which appears to be notable for the lack of immunosuppression, is the combination of vincristine and actinomycin D, as used in the treatment of Wilms' tumor.

The mechanisms by which classical cytotoxic agents induce T-cell depletion have not been well studied, but the effects are quite rapid. Within 1 day after initiation of cyclophosphamide therapy, there are already substantially reduced numbers of $CD3^+$ T cells in the peripheral blood that persist throughout the duration of therapy. Although classical models would suggest that alkylating agents primarily induce cell death through interference with cell division, chemotherapy induces a preferential depletion of naïve ($CD45RA^+$) $CD4^+$ T cells.[106] This subset has been shown to cycle at a diminished rate compared to memory ($CD45RO^+$) $CD4^+$ T cells,[100] suggesting that the lymphotoxic effect of these agents may not be dependent on cell cycling. Indeed, *in vitro* studies have shown that chemotherapy agents and radiation can induce spontaneous apoptosis in lymphoid cells, and clinical observations suggest that this may occur *in vivo* as well.[123]

With regard to other lymphocyte populations, B cells also sustain profound depletion in the context of dose-intensive multiagent chemotherapy. Generally however, this is accompanied by only modest reductions in serum IgG levels. In contrast, serum IgM and serum IgA levels undergo significantly greater depletion as a result of dose-intensive cytotoxic antineoplastic therapy. [113] NK cells, in contrast, appear to be relatively resistant to cytotoxic antineoplastic therapy, raising the possibility that they serve an important second line of host defense against viral pathogens in this setting and may be amenable to antitumor immunotherapeutic strategies, even in the face of chemotherapy-induced T-cell dysfunction.

Many of the current studies in this field have focused on changes in T-cell number, which are clearly a predominant feature of cytotoxic antineoplastic therapy–induced immunosuppression. There is also evidence to suggest, however, that important functional alterations may occur after cytotoxic antineoplastic therapy. After chemotherapy, we have observed a preponderance of activated T cells *in vivo*, which appear to have a heightened susceptibility for activation-induced programmed cell death.[124] Such functional alterations could significantly limit the capacity for response to antigen *in vivo*. Furthermore, restrictions in TCR repertoire diversity are likely to limit immune competence in hosts who have regenerated T cells predominantly via thymic-independent peripheral expansion. [125] Finally, recent studies have suggested that monocyte populations that are contained within peripheral blood stem cell harvests, and that frequently expand *in vivo* after cytotoxic antineoplastic therapy, may contribute to T-cell immunosuppression by the production of suppressive factors that inhibit T-cell function. [126],[127] Therefore, functional alterations in recovering T-cell and mononuclear populations may also contribute to cytotoxic antineoplastic therapy–induced immunosuppression.

In summary, the experience thus far suggests that although cancer patients have variable levels of immunosuppression at the time of presentation, the intensity of the antineoplastic therapy plays a central role in determining the risk for opportunistic complications. Clearly, such complications are most common in the setting of bone marrow transplantation; however, the use of immunosuppressive agents as part of dose-intensive regimens can lead to profound immunodeficiency in the non-BMT setting as well. The immunodeficiency induced by anticancer therapy appears to be primarily related to lymphocyte depletion and, perhaps most important, to CD4 depletion wherein increased depletion is associated with a higher incidence of opportunistic complications.

**T-Cell Regenerative Pathways**

When absolute lymphocyte counts are monitored after cessation of cytotoxic antineoplastic therapy, recovery to baseline values generally occurs within 3 months. NK-cell populations typically normalize immediately after cessation of therapy, [128] and these are followed by total B-cell populations, whose numbers generally normalize within 1 to 3 months.[129],[130] B cells often exhibit an overshoot such that supranormal values may be observed for a prolonged period after completion of cytotoxic chemotherapy. Restoration of total numbers of peripheral blood $CD8^+$ T cells generally occurs within 3 to 6 months after completion of cytotoxic antineoplastic therapy, with supranormal values for this subset also frequently observed during the initial phase of regeneration. [128] Such normalization of total lymphocyte counts belies the fact that recipients of cytotoxic antineoplastic therapy frequently experience prolonged CD4 lymphopenia. To understand the processes at work during this complex process of lymphocyte repopulation, we first review the various pathways of T-cell regeneration, with an emphasis on the implications of each, for the restoration of host immune competence.

The ontogenic or primary developmental pathway of T-cell development involves progeny of the pluripotent hematopoietic stem cells, which home to the thymus where they undergo an elaborate process involving expansion, differentiation, and selection. These newly produced cells are exported from the thymus bearing a naïve phenotype, and they subsequently travel throughout the peripheral lymphoid tissues awaiting encounter with antigen. Although a definitive marker for such thymic emigrants does not exist, they are contained within the $CD4^+CD45RA^+$ CD62hi subset.[131] Recently, a new method has been used to quantify recent thymic emigrants by employing polymerase chain reaction–based enumeration of cells that contain remnants of TCR rearrangement termed *TCR rearrangement excision circles*.[131] Thus far, however, available technology does not allow direct analysis of thymic emigrants because analysis of cell surface expression and intracellular TCR rearrangement excision circle expression cannot be performed simultaneously.

Importantly, T-cell populations can be regenerated in hosts in the absence of a thymus, primarily via a process that has been termed *peripheral expansion*.[132] Very simply, this involves the mitotic division of mature T cells themselves, thus increasing their cell number. Although peripheral expansion dramatically increases total body T-cell number, it is generally unable to completely normalize T-cell numbers, and it cannot restore TCR repertoire diversity. Indeed, because the process of peripheral expansion is driven by antigen encountered in the periphery, it is expected that contraction of the peripheral TCR repertoire will occur during the course of this process. Hence, it is not difficult to understand why thymic-dependent pathways are preferred for restoration of T-cell immune competence. When vigorous, the thymic-dependent T-cell regenerative pathway can both efficiently restore normal T-cell number and provide TCR repertoire diversity. When less vigorous, thymic-dependent pathways may still play an important role in the gradual replenishment of TCR repertoire diversity over time.

In the clinical setting, the degree of $CD4^+$ depletion induced by cytotoxic chemotherapy occurs in an age-independent fashion and is related to the intensity of

therapy. Importantly, however, the recovery of $CD4^+$ T-cell populations is highly age related, at least when comparing children to young adults. Here, recovery of $CD4^+$ T-cell populations 6 months after completion of cytotoxic antineoplastic therapy is inversely related to age in a more or less linear fashion such that children have brisk recovery, young adolescents show modest recovery, while older adolescents and young adults show poor CD4 recovery. [133] Furthermore, children typically show brisk recovery of the naïve ($CD45RA^+CD4^+$) subset coincident with recovery of total $CD4^+$ T-cell populations, and these rises are temporally associated with radiographic evidence of thymic enlargement post-chemotherapy (Fig. 6-10). Importantly, the gradual diminution in the ability to recover CD4 populations throughout childhood suggests that rather than a precipitous drop in thymic function occurring at the time of puberty, more likely there is a gradual diminution in thymic function that parallels the gradual reduction in thymic mass that is known to occur in humans during the first two decades of life. In contrast, adults generally show a slow, variable rise in $CD4^+$ populations over the first year after cessation of antineoplastic cytotoxic therapy. In this setting, $CD4^+$ cells predominantly display the memory ($CD45RO^+CD4^+$) phenotype.[124] During subsequent years, it is not uncommon to see gradual rises in naïve $CD45RA^+CD4^+$ cells in adults as well, although obvious radiographic evidence of thymic enlargement is rarely observed. Therefore, thymic-dependent pathways appear capable of restoration of $CD4^+$ T cells in children during the first 6 months after cessation of antineoplastic chemotherapy, whereas $CD4^+$ T-cell recovery in adults is dependent on the relatively inefficient thymic-independent pathways and perhaps variable degrees of low-level thymopoiesis ( Fig. 6-9). The clinical implication is that although children typically experience a relatively short period of immunosuppression associated with cytotoxic antineoplastic therapy, this period may be prolonged in older adolescents and adults. Importantly, however, if children are experiencing complications that may further impair thymic function, such as GVHD, they experience prolonged $CD4^+$ depletion similar to that observed in adults.[134] Similarly, prolonged CD4 depletion in excess of 30 years has been observed in recipients of mediastinal irradiation for Hodgkin's disease, suggesting that thymic function may be irreversibly impaired after local radiation therapy. [135] Finally, even in children, the initial months after completion of chemotherapy are likely predominated by thymic-independent pathways as recovery generally takes 3 to 6 months even in the youngest of patients.

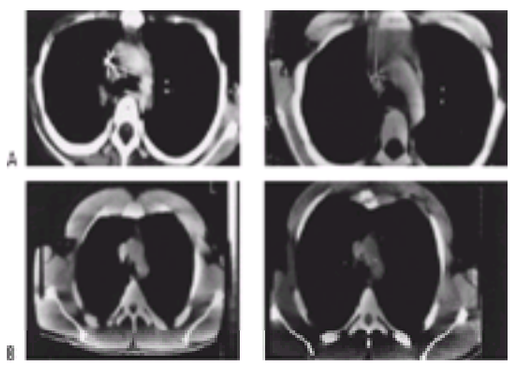

**FIGURE 6-10.** Thymic rebound after intensive chemotherapy. **A:** A contrast-enhanced computed tomography scan taken pre-therapy and 6 months after completion of dose-intensive chemotherapy in an 11-year-old treated for large cell lymphoma. Enlargement of the thymic shadow compared to baseline is observed. This was associated with a rise in peripheral blood total $CD4^+$ T-cell counts as well as $CD45RA^+CD4^+$ T cells. Biopsy of the thymus at this time point revealed normal thymic tissue. The hyperplastic organ subsequently returned to baseline size over the ensuing months. In contrast, enlargement of the small amount of thymic tissue present at baseline is not observed in the 19-year-old patient shown in **B**. Left: Baseline before chemotherapy. Right: 6 months after completion of chemotherapy. (From Mackall CL, Fleisher TA, Brown MR, et al. Age, thymopoiesis, and CD4+ T-lymphocyte regeneration after intensive chemotherapy. N Engl J Med 1995;332:143–149, with permission.)

With regard to $CD8^+$ T cells, the story is complicated by the existence of multiple $CD8^+$ T-cell subsets, many of which recover in an age-independent fashion after cessation of cytotoxic chemotherapy. In a study of children recovering from intensive chemotherapy, it was noted that unlike $CD4^+$ cells, no such relationships exist between age, thymic enlargement, and $CD8^+$ T-cell regeneration after chemotherapy.[128] Typically, within 3 months after cessation of cytotoxic chemotherapy, total $CD8^+$ numbers generally return to baseline regardless of patient age. Careful analysis of such populations, however, show that the bulk of the $CD8^+$ T cells contained within the recovered populations are atypical and represent the expansion of a normally minor subset of $CD8^+$ T cells that lack the CD28 co-receptor. Indeed, recovery of the $CD8^+CD28^+$ subset generally does not occur until approximately 1 year after cessation of therapy. The $CD8^+CD28^-$ cells that tend to predominate after chemotherapy are poorly responsive to mitogenic stimuli and may function primarily as negative regulatory or suppressor populations. [136,137] Several lines of evidence also suggest that such cells may be derived via thymic-independent pathways.[138,139] Therefore, although total $CD8^+$ T-cell numbers normalize rapidly after cessation of cytotoxic chemotherapy, it appears that abnormalities in the $CD8^+$ arm of the immune system also persist for a prolonged period after cessation of cytotoxic chemotherapy due to the expansion of a normally minor subset with abnormal functional capacity.

Therapeutic modalities for enhancing immune reconstitution are sparse at the present time. Although GM-CSF has been reported to improve immune reconstitution for patients treated with autologous BMT for lymphoma,[140] we observed prolonged T-cell depletion post-chemotherapy and post-BMT despite the use of GM-CSF in our patient populations.[113,133] Other potential cytokines include the use of IL-2, which has been shown to improve immune reconstitution in murine models and to increase $CD4^+$ counts in patients with HIV infection.[141] Alternatively, the provision of large numbers of cryopreserved autologous peripheral blood (mature) T cells is predicted to enhance immune reconstitution by contributing to thymic-independent peripheral expansion. It appears, however, that such T cells may be limited in their capacity to contribute to immune reconstitution if they are harvested after the initiation of chemotherapy, perhaps due to a susceptibility to programmed cell death.[113,124] Current studies are under way to ascertain whether the combination of systemic IL-2 therapy and infusion of chemotherapy-naïve mature T cells can rapidly reconstitute T-cell populations after chemotherapy. Future approaches are likely to involve the administration of agents that can modulate programmed cell death in expanding T cells, including the use of IL-7, which has been shown to be a potent modulator of immune reconstitution in animal models. [142]

## CANCER AS AN IMMUNE TARGET

Although the principal role of the immune system is protection from infectious pathogens, oncologists have sought to manipulate immunity to control cancer for at least 100 years. Although these efforts have yet to be translated into clinically effective therapies for the majority of human tumors, many lines of evidence suggest that immunotherapy for neoplastic disease may ultimately become a therapeutic reality. In this section, we review work spanning several decades that forms the basis for this hypothesis and emphasize current approaches for targeting human tumors, with a particular focus on issues of relevance to pediatric tumors. Specific immunotherapies and clinical trials are discussed in detail in Chapter 14.

### Animal Models

Early investigators used mouse models to study the ability of hosts to generate immune responses toward tumors. By necessity, this involved transplanting tumors from one mouse to another to evaluate the ability to reject transplanted tumors. To better evaluate potential mechanisms, spontaneously arising and experimentally induced neoplasms in animals were transplanted to other animals of the same species to evaluate immunization protocols and potential immune responses. Initially, the success of this approach was thought to be overwhelming. Virtually all transplanted tumors, if transplanted in small enough aliquots, were readily destroyed by immunologic mechanisms. Although severely immunodeficient or irradiated mice did not control even small transplanted tumor fragments, immunologically intact mice could readily destroy most allogeneic transplanted tumor fragments. However, these studies were undertaken before the development of an understanding of allogeneic immune responses, which are fully capable of eradicating tissues (either benign or malignant) that are transplanted between genetically disparate hosts within the same species. Indeed, as described by Sir Peter Medawar in his review of this literature in 1965, he noted the “immunologists who attempted to use transplantation to study tumors were instead using tumors to study transplantation.” Regardless, these studies did provide proof of the principle that immune effectors can eradicate tumor cells, although even in this setting this could be overwhelmed by large tumor burdens. Furthermore, these studies pointed out that a primary requirement for the generation of clinically relevant immunotherapies is the identification of antigens that could be used to target the immune response to syngeneic tumors.

Fortunately for the sake of scientific clarity, the fallacy of this approach of transplanting tumors was rapidly clarified. The ability to reject allogeneic transplanted tumors was readily documented by Gorer, Snell, and others to be the result of recognition of genetically inherited distinct transplant antigens (reviewed elsewhere[143,144,145] and [146]). Major and minor histocompatibility antigens expressed on all tissues of the tumor donor were the targets of T-cell recognition when the host rejected the transplanted allogeneic tumor. Indeed, it was the very weak to nonexistent immune response detected against syngeneic tumors of identical

histocompatibility type that enabled Gorer and Snell to identify the genes responsible for allograft rejection reactions and to generate congenic resistant strains differing only for these transplant-related genes. Despite the importance of these studies for clarification of the basic biology of transplant rejection, these results suggested that immune responses to syngeneic tumor were negligible.

### Tumor-Specific Transplantation Antigens

Independent studies evaluated another approach wherein the development of tumors within an individual mouse could be induced using viruses or chemical carcinogens. Prehn and Main induced murine tumors with the carcinogen methylcholanthrene (MCA). These tumors were fatal in the tumor-bearing host and were fatal when transplanted to syngeneic animals owing to local tumor growth and invasion. These tumors, unlike most clinical cancers, did not metastasize. Animals bearing a rapidly growing transplanted syngeneic tumor could have the tumor entirely resected surgically and be rendered tumor free. Such animals would survive tumor free. On rechallenge with the same tumor that had been growing in them originally (and that would have proved fatal), a clear immunologic response was mediated against the tumor (Fig. 6-11). Provided the rechallenge with the initial tumor used a small enough tumor transplant, the immune response could completely reject the transplanted tumor and provide protection.[147] This immune-mediated rejection was used as evidence for recognition of tumor-specific transplant antigens (TSTAs) that could not have been alloantigens because the tumors were derived from syngeneic animals.[144] Similar results were obtained with most experimental carcinogens used to cause chemically induced murine cancers. Although the nature of the TSTA in these chemical carcinogen-induced tumors remains somewhat uncertain, these data provided functional evidence for TSTA distinct to individual tumors.

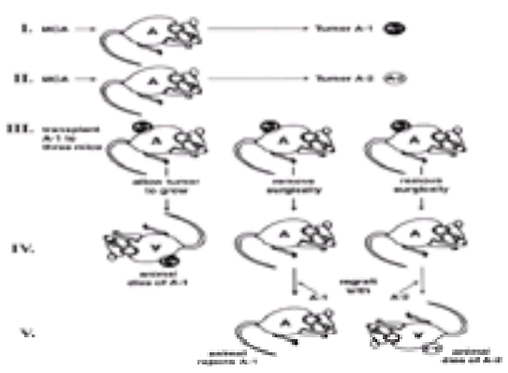

**FIGURE 6-11.** Rejection of chemically induced tumors. All mice shown (A) are strain AA mice (homozygous, syngeneic animals, with two identical strain A alleles at each genetic locus). The animal at the top in line I was treated with methylcholanthrene (MCA) to generate a fibrosarcoma-designated A-1. The animal in line II was treated with MCA and tumor A-2 developed. Line III shows grafting of a small amount of tumor A-1 onto three syngeneic AA animals. This tumor will grow progressively, and the animal will die (first mouse on line IV) if it is not removed. If the tumor is removed surgically, both animals will survive. If one of these mice is regrafted with some of tumor A-1, an immune reaction will develop and destroy tumor A-1 (left hand animal in line V). However, if the mouse is regrafted with a separate tumor (even a separate syngeneic tumor, such as A-2) it will not be rejected and will grow progressively (animal on R in line V). [Adapted from Prehn RT, Main JM. Immunity to methylcholanthrene-induced sarcomas. JNCI 1957;18:769; and from Sondel, PM, Rakhmilevich AL, DeJong JLO, Hank JA. Cellular immunity and cytokines. In: Mendelsohn J, Howley PA, Israel MA, Liotta LA, eds. The molecular basis of cancer. Philadelphia: Saunders, 2001 (in press).]

For virally induced tumors, research by Gross and by several other laboratories documented analogous TSTAs.[145] One difference, however, was that syngeneic tumors induced by the same virus in the same strain appeared to show cross-reactive TSTA with one another, suggesting a shared antigen on the surface of tumors caused by the same virus.

Three decades of *in vitro* and *in vivo* work with these experimental models have documented that the principal responding component of the immune system in all of these tumor rejection models are the T cells.[148] CTL and Th lymphocytes are typically involved and are most effective when working simultaneously against a transplanted tumor. In some model systems, the immunoprotective effect can be transferred by injecting T cells from a single-cell–derived clone (i.e., all cells in the clone express the same TCR gene rearrangement pattern), which provides specific recognition of the transplanted tumor.[149] In such models, administering IL-2 (Fig. 6-12) to the adoptively transferred tumor-bearing host can enable further *in vivo* growth and activity of the tumor-specific T cells.[150] Molecular analyses in some of these systems have clarified the nature of the tumor antigens recognized. For example, in the potent protective T-cell response to the murine leukemia caused by the Friend leukemia virus, a specific class II MHC molecule on the murine antigen-presenting cells presents a peptide contained within the virus's coat envelope protein (ENV) controlled by the viral ENV gene. These ENV-specific Th cells collaborate with CTL that recognize a nuclear peptide controlled by the GAG viral gene as expressed on the leukemia cell surface through its presentation in the groove of a specific class I MHC allele.[151] These animal models have formed the basis for a large number of ongoing preclinical efforts by many laboratories to identify appropriate molecules on human tumors, which could be used as T-cell targets. In the next section, we review current approaches for identifying and targeting such human tumor antigens.

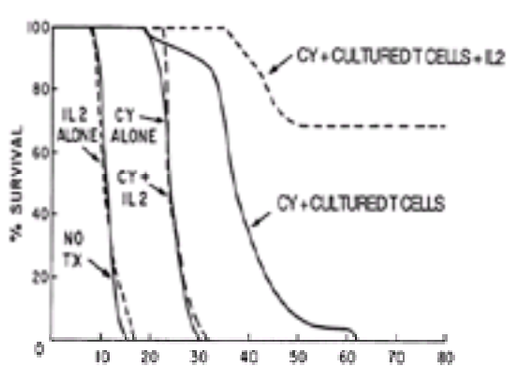


**FIGURE 6-12.** Efficacy of purified interleukin-2 (IL-2) *in vivo* as an adjunct to long-term cultured T lymphocytes in adoptive chemoimmunotherapy. C57BL/6 mice inoculated intraperitoneally with $5 \times 10^6$ FBL-3 on day 0 received no therapy (no Tx), treatment with 80 U per day of IL-2 on days 5 through 9 (IL-2 alone), treatment on day 5 with cyclophosphamide (CY) at a dose of 180 mg per kg (CY alone), treatment with CY on day 5 plus IL-2 on days 5 through 9 (CY + IL-2), treatment on day 5 with CY and $5 \times 10^6$ long-term cultured T lymphocytes immune to FBL-3 (CY + cultured T cells), or treatment on day 5 with CY plus C57aFBL (cultured) plus IL-2 on days 5 to 9 [CY + C57aFBL (cultured) + IL-2]. (From Cheever MA, Greenberg PD, Fefer A, Gillis S. Augmentation of the antitumor therapeutic efficacy of long-term cultured T lymphocytes by in vivo administration of purified interleukin-2. J Exp Med 1982;155:968, with permission.)

### Identification of T-Cell Tumor Antigens

#### *Tumor-Reactive T-Cell–Defined Tumor Antigens*

Careful histologic analysis of a variety of human tumors reveals the presence of a lymphocytic infiltrate in a large number of tumor types. Investigators studying immune responses to malignant melanoma and renal cell carcinoma have attempted to identify the targets of these so-called tumor-infiltrating lymphocytes (TILs). Identification of tumor antigens by cloning the targets of TILs rests heavily on the assumption that tumor growth evokes a natural, endogenous immune reaction, at least a portion of which is directed toward tumor-associated molecules. Most commonly, this approach begins with the extraction of TILs from immune-responsive tumors such as malignant melanoma or renal cell carcinoma. Individual T-cell clones that are capable of lysing autologous tumor targets are generated, and the HLA-presenting allele on the target cell is identified. To identify the molecular target of the tumor-specific cytolytic T cells, complementary DNA libraries from the autologous tumor are cloned into cell lines that express the HLA-presenting allele. Identification of those clones that result in lysis is carried out sequentially until the

gene that is targeted by the T-cell clone is identified. [152,153]

This approach has led to the identification of several melanoma-associated tumor antigens that, when used as immunogens, have been shown to induce antitumor activity in clinical trials. [154,155] In general, the targets identified using this approach can be grouped into three main families. The first are the so-called cancer-testis antigens, which include molecules of the MAGE, BAGE, GAGE, PRAME, PAGE, and XAGE families. They are called *cancer-testis antigens* because they are expressed in a wide array of cancer tissues but are restricted in their normal tissue distribution to testes. Because human germ cells lack class I expression and are generally inaccessible to the immune response, it is hypothesized, and has now been experimentally proven, [156] that immune responses directed toward such antigens would spare all normal tissues. Importantly, although many molecules within the cancer-testis antigen family were initially identified using TILs derived from malignant melanomas, most of the antigens identified within this family are also expressed in other, nonrelated tumors. Presumably, this is related to demethylation of cancer-testis antigen promoters that occurs commonly in neoplastic tissues and that can induce the expression of these molecules in normal tissues. [157,158] Therefore, study of the targets of natural immune responses, particularly immunoresponsive tumors, such as malignant melanoma and renal cell carcinoma, has surprisingly led to the identification of potential public tumor antigens that could also be used as targets for immune response induction in a variety of histologically unrelated tumors.

With regard to pediatric tumors, the GAGE-1 molecule has been identified in 82% of neuroblastomas and 100% of stage IV tumors. [159] Indeed, the use of GAGE-1 as a target for monitoring minimal residual disease in neuroblastoma has been pioneered by Cheung and colleagues. [160] Although studies are limited in other pediatric tumors, GAGE-1 has also been seen in five of five Ewing's sarcomas,[159] and GAGE-1,2 is observed in 25% of other sarcomas. [161] GAGE-3,6 molecules are expressed in 78% of pediatric glioblastomas and 47% of medulloblastomas. [162] Molecules of the MAGE family are also expressed in pediatric tumors with reports of 50% to 80% of neuroblastomas expressing at least one of the four identified MAGE genes. [160,163,164 and 165] Osteosarcomas express MAGE-1, -2, -3, and -6 in approximately 50% of cases.[166] In one report, 11% of pediatric glioblastoma multiforme tumors and 60% of medulloblastomas expressed MAGE-2 whereas 13% of medulloblastomas expressed MAGE-3 and -6.[162] PRAME, another molecule identified by screening T cells reactive against autologous melanoma, has been found in a wide variety of human cancers, including sarcomas, head and neck tumors, and renal carcinomas.[167] This molecule is also observed on approximately 25% of acute leukemia samples, with 100% of those expressing the t(8;21) also expressing the PRAME molecule.[167] Using a computer-based approach to identify homologues of the GAGE family of genes, Brinkmann et al.[168] recently identified a new family, termed *XAGE*. XAGE-1 and XAGE-2 proteins were observed in Ewing's sarcoma and alveolar rhabdomyosarcoma. Therefore, identification of targets of T cells reactive against unrelated tumors has allowed the identification of multiple families of genes that occur across a wide spectrum of malignancies, some of which have been observed in pediatric cancers. Clearly, a great deal more work is required to fully characterize the range and extent of expression of these molecules in pediatric tumors. It is clear, however, that peptides derived from these molecules display significant binding to several common HLA alleles, raising the possibility that they could serve as targets for T-cell–mediated immune responses in some pediatric tumors.

The second family of molecules identified using this approach has been termed *melanocyte differentiation antigens*, as they are expressed on melanoma cells and also on normal melanocytes. These molecules include gp100/pmel17, melan-A/Mart-1, tyrosinase, TRP-1, and TRP-2. [161] Although expression of these antigens on cancers appears to be restricted to melanoma, their identification provided an important conceptual advance that is potentially of interest for the development of a wide variety of immune-based cancer therapies. Immunologic dogma has generally held that tolerance toward “self” molecules (such as differentiation antigens) occurs early in life, and that attempts to generate immune responses to such proteins would likely be unsuccessful. In clinical trials using immunization strategies targeting melanocyte differentiation antigens, however, not only have clinical antitumor responses been observed, but they are frequently associated with vitiligo, thereby providing evidence that such responses are also directed toward normal melanocytes. [169,170 and 171] These results provided the important observation that although immune tolerance toward “self” proteins is normally maintained in healthy individuals, immunotherapies can break tolerance and induce immune responses directed toward proteins that are not tumor specific, but rather tumor associated (i.e., also expressed on normal cell populations). The corollary of this observation is that autoimmunity is a potential risk of any antitumor immunotherapy directed against molecules that are not tumor specific; therefore, clinical complications related to autoimmunity must be monitored closely as such clinical trials evolve. Furthermore, the most attractive tumor-associated targets for immunotherapy development are those for which expression in normal tissues is limited to “nonvital” tissues such as melanocytes. Clearly, a better understanding of the mechanisms by which host T cells are tolerized to tumor antigens *in vivo* remains an important area of research and is central to generating immune therapies aimed at tumor-associated molecules.[172]

With regard to pediatric malignancies, it remains a possibility that classical oncofetal antigens or other molecules expressed during normal fetal development and also within neoplastic tissues could serve as targets for a T-cell–mediated immune response. Examples might include alpha-fetoprotein in hepatoblastoma, PAX-3 in rhabdomyosarcoma,[173] or chromogranin A or neuropeptide Y in neuroblastoma.[174,175] Potential drawbacks of targeting such molecules include the possibility that the selection against self-reactive T-cell clones of high avidity, which does occur early in life, results in a T-cell repertoire containing only T cells of relativity low avidity for such differentiation antigens. Indeed, TCRs with low avidity have been shown to function less well in clearing viral infection and tumors *in vivo*.[176,177] Furthermore, because expression of both cancer-testis antigens and differentiation antigens are generally not critical for viability of the tumor cell, the possibility of tumor escape by immune selection of antigen negative clones remains a real possibility. [155]

More recently, tumor antigens identified via the study of TILs have also included mutated forms of normal “self” molecules. Although the normal “self” molecule should not be recognized immunologically, the specific mutation of the molecule within the tumor allows the mutated form to be recognized as “foreign.” In one example, a mutated form of b-catenin was identified via subtractive cloning of melanoma-reactive TILs. [178] Similarly, a mutated form of the cyclin-dependent kinase, CDK-4, has been identified as a target of TILs.[179] Such molecules are particularly attractive as potential targets as they directly contribute to the neoplastic state and are therefore theoretically less susceptible to further mutation, hence diminishing the potential for immune escape by selection of antigen-negative targets. Indeed, a great deal of ongoing work by many laboratories currently using directed approaches to target such oncologically relevant, tumor-specific mutations is discussed in the following section.

### *Induction of Immune Responses Toward Independently Characterized Tumor-Specific Molecules*

The second approach for identifying potential targets for T-cell–based therapy of cancer rests heavily on the assumption that the rate-limiting factor in the generation of natural, endogenous immune responses to tumors is not the dearth of unique or aberrant molecules expressed by tumors, but rather the absence of secondary, costimulatory signals that are critical for activation of the immune system. A central theme in basic immunology holds that for T cells to become activated toward a particular antigen, two requirements must be met. First, the antigen must be presented as a peptide by a MHC molecule in a form that is recognizable to the T cell—signal one. Simultaneously, however, a second signal must be provided to the T cell, preferably via the same cell delivering signal one. The second signal is required to fully activate the signaling pathway within the T cell. [35] Because tumor cells in general lack costimulatory molecules that are capable of delivering the second signal, it remains possible that a great many antigens are presented on the surface of tumors, but in the absence of the second signal, the result is T-cell tolerance, rather than T-cell activation. Based on this model, one of the most critical components of immunotherapy development is the provision of adequate costimulation during the induction phase of the immune response. Currently, this can be achieved through immunization using dendritic cells, which were discussed earlier in this chapter in the section Critical Role of Costimulation: Antigen-Presenting Cells. Based on this model, many tumor immunologists are currently attempting to target tumor-specific molecules that have been identified during the course of investigations into the molecular mechanisms of neoplastic transformation. Although most of these molecules are intracellular, rather than cell surface molecules, it is now clear that intracellular molecules can serve as potential targets for T-cell–mediated immune responses as a result of the normal processes of protein breakdown, antigen processing, and presentation of antigenic peptides by surface MHC molecules.[27]

Examples of tumor-specific mutations that have been targeted immunologically include mutations in the Ras protein in which immune responses to mutant Ras peptides have been shown in mouse and human models.[180,181] Ras mutations occur commonly in adult lung and gastrointestinal carcinomas, whereas in pediatrics, Ras mutations are much less common. Similarly, experimental models have shown successful immunologic targeting of mutant p53, which is known to occur in a variety of adult tumors.[182,183 and 184] The difficulty associated with immune targeting of p53, however, is the requirement for sequencing and producing individualized p53 peptides for each patient because alterations in this molecule are highly variable.

In pediatric oncology, characteristic chromosomal translocations occur in a variety of tumors and provide potential targets for T-cell–mediated immune responses. This is because peptides that span the breakpoint region of the translocation represent novel epitopes that do not exist in normal tissues and hence may be susceptible to immune targeting (Fig. 6-13). Recent studies have attempted to target the breakpoint regions of the t(2;13) and the t(11;22) found in alveolar rhabdomyosarcoma and the Ewing's sarcoma family of tumors, respectively. For this approach, 15 to 18 amino acid peptides were synthesized that spanned the breakpoint region of the t(2;13) and the t(11;22) type 1 and type 2 in Ewing's sarcoma. Using computer-based models, several of the nonamers derived from these peptides were predicted to bind to mouse and human APC. The peptides were then pulsed onto murine APCs, and the irradiated pulsed APCs were administered to mice. Animals sensitized in this manner generated cytolytic T cells that were specific for the peptide and also specifically recognized and killed CT26 adenocarcinoma

cells transfected to express the full-length t(2;13) fusion protein. Therefore, these results demonstrated that breakpoint region peptides could be processed and presented on the cell surface in the context of murine MHCs. Furthermore, immunized mice were protected *in vivo* against a subsequent tumor challenge with transfected CT26 adenocarcinoma cells, and adoptive transfer of immunized cells led to antitumor responses in hosts bearing transfected CT26 adenocarcinoma cells. Based on this preclinical work, current clinical trials are under way to test whether peptides derived from the breakpoint region of t(2;13) and t(11;22) are capable of eliciting immune responses and antitumor effects in high-risk patients with alveolar rhabdomyosarcoma and Ewing's sarcoma.


**FIGURE 6-13.** Generation of a tumor-specific T-cell epitope as a result of a chromosomal translocation. Shown is the amino acid sequence of the breakpoint region of the t(2;13)(q35;q14) found in alveolar rhabdomyosarcoma. The peptides derived from the wild-type PAX3 and FKHR genes would be predicted to be recognized as self-peptides and therefore ignored by the immune system. However, the generation of the chromosomal translocation results in a series of eight breakpoint region peptides (designated bp 1 to 8) nine amino acids in length (optimal size for binding to major histocompatibility complex class I), which could potentially be recognized as novel tumor-specific epitopes by T cells.

Other chromosomal translocations that have been shown to provide peptide sequences at the breakpoints that can serve as targets for immune-mediated responses include the t(9;22), which is seen in essentially all patients with chronic myelogenous leukemia and approximately 5% of pediatric patients with acute lymphoblastic leukemia.[185] Peptides derived from the breakpoint region of the t(9;22) can induce immune responses in healthy people, as well as in chronic myelogenous leukemia patients, and such T cells have been shown to be capable of lysing chronic myelocytic leukemia targets that express the HLA-presenting allele. Similar results have been observed for the t(12;21), which occurs in approximately 25% of acute lymphocytic leukemia patients.[186] Recent studies have also shown evidence for binding of breakpoint peptides derived from t(X;18) in synovial cell sarcoma as well as t(11;22) in desmoplastic small round cell tumor to common HLA alleles, suggesting that these peptides could also potentially serve as immunogens in these tumors.

Although targeting such eminently tumor-specific molecules as chromosomal translocations is attractive due to their penultimate specificity, potential drawbacks include the relatively narrow immune response that is likely to be generated toward the limited number of epitopes that make up the breakpoint region. Similarly, because peptide-based therapies require HLA binding of individual peptide epitopes, it is possible that sufficient HLA binding of breakpoint peptides will only exist for a limited number of HLA alleles. This limitation is particularly relevant to pediatric tumors that are rare enough in the general population to limit the practicality of conducting trials and developing therapies limited to patients with unique HLA alleles (as has been possible for testing of these vaccine strategies in the more common malignancies in adults with cancer).

***Whole Tumor Cell Vaccination***

A third approach for targeting T cells toward tumor cells is to exploit the antigen processing and antigen-presenting capacities of professional APCs by supplying the entire array of antigens that may be contained within the whole tumor cell to the APC. This approach rests heavily on the assumption that the HLA makeup of each individual is a primary factor in determining which molecules are most immunogenic for any individual. Therefore, it may be less important to identify individual antigens than to devise methods to allow each individual's own antigen-processing machinery to present HLA-binding molecules present within the tumor to the individual's immune system. This approach also theoretically would induce the development of a broad, polyclonal immune response rather than focusing the immune response on one particular antigen, thus potentially amplifying the number of lymphocytes capable of responding to the tumor and diminishing the likelihood of tumor escape via immune selection. Indeed, the clinical use of whole tumor cell vaccines would not require the identification of individual target antigens for individual tumors and even assumes that different molecules will prove to be more or less immunogenic in different individuals. Such an approach could be particularly useful in pediatric oncology wherein the practical issues surrounding the development of distinct antigens as clinical products for each HLA allele in rare tumors are substantial.

Currently, there are a variety of means under study to present the complete antigenic array of a given tumor cell to T cells via dendritic cells. Fusing tumor cells to autologous dendritic cells has recently allowed the tumor-dendritic cell hybrid to be used as an effective vaccine for inducing antitumor responses in patients with renal cell cancer.[187] Another method comes from a recently published report wherein it was shown that dendritic cells preferentially ingest apoptotic and necrotic cells and can subsequently present antigenic peptides derived from such cells to autologous T cells.[41,42] Theoretically therefore, apoptotic necrotic tumor cells could be fed to dendritic cells as immunogens. Similar results have been obtained using RNA that has been fed to dendritic cells wherein the induction of tumor-specific CTLs has been shown.[188,189] This technique has the advantage of requiring only limited amounts of tumor tissue in cases in which cell lines are not available, which could be particularly pertinent to the development of such therapies in pediatrics. Tumor lysates [190] as well as acid-stripped antigenic peptides have also been shown to be capable of inducing tumor-specific immune responses.[191] Tumor-cell–derived heat shock proteins, which presumably function as molecular chaperones within the tumor cell and are thus a rich source of endogenously processed peptides, can also potently induce tumor-specific immune responses by providing an array of immunogenic peptides to autologous T cells.[192]

These advances in our understanding of the function of T cells, antigen processing, and presentation by APC, and the molecules that are selectively expressed on human tumor cells, have set the stage for the potential clinical development of effective active immunotherapy. Current goals of research in this area are to identify the optimal approaches to induce a patient's own T cells to selectively recognize and destroy autologous tumor cells. Separate immunotherapy strategies are based on tumor recognition by cells of the innate immune system and by Ig molecules produced by B cells.

**Antitumor Potential of the Innate Immune System**

Cells of the innate immune system are not as effective at mediating tumor cell destruction as are activated, antigen-specific CTLs. However, when activated, certain cells of the innate immune system, particularly NK cells and macrophages, can mediate potent destruction of tumor cells *in vitro* and in animal models. Thus, several clinical strategies have been pursued whereby activators of the innate immune system (IFNs or IL-2 to activate NK cells, muramyl-tri-peptide-phosphatidyl-ethanolamine to activate macrophages) have undergone clinical testing with some signs of clinical benefit in treating spontaneously arising tumors in companion animals or in patients. However, the actions of these activated cells do not seem to be sufficiently tumor specific. A variety of "nonspecific" toxicities (e.g., fever, rash, capillary leakage, and rigors) are associated with these approaches, and may reflect the systemic effects of the downstream "cytokine storm" induced by these activators of the innate immune system. More recent approaches are trying to utilize T- or B-cell tumor recognition mechanisms to enhance the antitumor capabilities of the innate immune system (see following section).

**Tumor-Specific Selective Antibodies**

Although the T-cell component of the immune system appears to be pivotal in many experimental and therapeutic immune responses to tumors, efforts directed at B-cell reactivity to tumor have also been pursued. For several human tumors, particularly melanoma, renal cell carcinoma, neuroblastoma, and head and neck cancers, biochemical separation of molecules found in human patient sera can demonstrate, in some patients, Ig molecules or immune complexes that appear to react with the patient's autologous tumor.[193] The possibility that these molecules may be mediating an antitumor effect or somehow masking the tumor surface from cellular immune recognition and destruction has been suggested, and additional data are needed. Nevertheless, the presence of human antibodies against human tumors promotes the possibility of generating antibody molecules that recognize tumor-specific or tumor-selective molecules to enable their use in the preferential binding *in*

*vivo* to tumor cells.

Since the creation of monoclonal antibodies in 1975 ( Fig. 6-6), labs worldwide have been immunizing mice with human tumor tissue and screening for antibodies that specifically recognize human cancer cells, but not any normal human tissues. Although rare antibodies of this type (“tumor specific”) have been described, most antibodies produced by this approach have been shown to recognize both neoplastic and normal human tissues. Some of these, which may be clinically useful, recognize molecules that are highly overexpressed on certain human tumors and expressed weakly on only a small histologically distinct subset of normal human tissues. Examples of such tumor-selective antigens that have been important in clinical pediatric oncology are: the CD19 molecule expressed on pre-B leukemia and lymphoma, and on certain precursor B cells; the CD33 molecule found on acute myeloid leukemia cells, as well as certain normal myeloid precursors; and the GD2 disialoganglioside, which is overexpressed on neuroblastoma and most osteosarcomas, but also expressed weakly on peripheral nerves. [194] Multiple molecular modifications of these and other similar tumor selective monoclonal antibodies are being pursued for diagnostic and therapeutic purposes. These include *in vitro* purging of tumor cells from hematopoietic stem cell and several strategies involving direct *in vivo* administration of these tumor-reactive antibodies.

The infusion of tumor-reactive antibody without directly activating antitumor recognition by the host's own immune system has been designated “passive” immunotherapy, to differentiate it from the “active” immunotherapy directed at inducing the host's own immune system to recognize the cancer directly. In fact, there may not be such a clear distinction between these active and passive approaches, as both may be working together. [195] For example, 35 years ago, Jerne proposed an “immune network hypothesis” whereby any immune response turns on a compensatory regulatory response. For example, in an immune response to a tumor antigen (e.g., GD2), an antibody against GD2 may be produced ( Fig. 6-14). This is designated as “antibody-1” (Ab-1). The unique peptide sequence of that Ab-1's antigen-binding component (generated through the somatically derived Ig gene rearrangement process) can be considered as an antigen itself for the immune system and is designated the “idiotype” of the Ab-1 molecule. Jerne proposed that this could induce the immune system to create an antiidiotype antibody (Ab-2), which recognizes the idiotype as foreign. Because the antigen-binding portion of the anti-GD2 antibody can specifically interact with the GD2 molecule, and can also specifically interact with the Ab-2 molecule, this implies that the antigen-binding portion of the Ab-2 molecule and the GD2 molecule may have some structural similarity. This concept becomes even more complex when considering the host's immune response against the unique peptide sequence of the antigen-binding portion of the Ab-2 molecule. The anti-Ab-2 molecule (in early descriptions referred to as an *anti-antiidiotypic antibody*) made by the host should recognize the antigen-binding portion of Ab-2 (which has molecular structural similarity to the GD2 itself); thus, the Ab-3 molecule may have direct antitumor recognition capabilities. Sufficient data in a variety of systems have now documented the validity of this concept. Thus, clinical trials providing passive treatment to patients with antitumor antibody (i.e., Ab-1) suggest antitumor effects may be influenced by the induction of Ab-3. [196] Furthermore, separate clinical trials are intentionally treating patients with antiidiotypic antibodies (i.e., Ab-2) to induce an Ab-3 response. [195] In such trials, the Ab-2 molecule is actually functioning as the antigenic component of a “tumor antigen vaccine.” Furthermore, it seems that some patients being “immunized” with Ab-2 molecules are also generating T-cell responses against the initial tumor antigen that had been recognized by the Ab-1 that was the “target “ of the Ab-2 molecule.

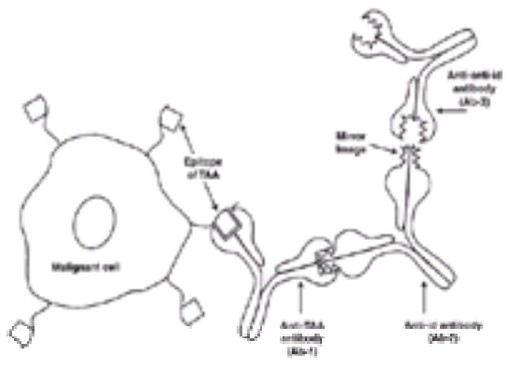


**FIGURE 6-14.** Mimicry of tumor-associated antigens (TAA) by anti-id antibodies. An epitope of a TAA is shown schematically by the box attached to a glycoprotein on the membrane of the tumor cell. A monoclonal antibody (Ab) was made in mice against this TAA. This anti-TAA antibody (Ab-1) has antigen-binding ends (Fab) that allow tight binding of the TAA epitope. When Ab-1 is used as an immunogen, it can induce an antibody directed against it. This antiidiotypic antibody (Ab-2) has antigen-binding sites that can bind to the antigen-binding sites of Ab-1. As such, the antigen-binding sites of Ab-2 may interact with the antigen-binding sites of Ab-1 in the same way that the antigen-binding sites of Ab-1 interact with the TAA itself. Thus, the antigen-binding sites of Ab-2 and the TAA are both recognized by Ab-1, and the antigen-binding sites of Ab-2 may be immunologically similar in structure to the TAA of the malignant cell. This similarity in structure is referred to as *internal image*. If the Ab-2 molecule is recognized as an antigen, the antibody directed against it is an “anti-anti-id antibody” (Ab-3). As the Ab-3 recognizes the internal image of the TAA found on Ab-2, the Ab-3 antibody may directly recognize the TAA itself. This is the rationale behind using an anti-id (Ab-2) to induce an immune response (Ab-3) able to recognize a TAA. [Adapted from Mittelman A, Wang X, Matsumoto K, Ferrone S. Anti-idiotypic response and clinical course of the disease in patients with malignant melanoma immunized with mouse anti-idiotypic monoclonal antibody MK2-23. Hybridoma 1995;14:175–181; and from Sondel PM, Rakhmilevich AL, DeJong JLO, Hank JA. Cellular immunity and cytokines. In: Mendelsohn J, Howley PA, Israel MA, Liotta LA, eds. The molecular basis of cancer. Philadelphia: Saunders, 2001 (in press).]

### Combined Approaches

Clinically effective antitumor immunotherapy remains a goal not yet fully achieved, despite many effective preclinical strategies and several promising clinical approaches currently being pursued. As we obtain a better molecular and cellular understanding of the immune system, and of the tumors we wish to eradicate through immune recognition and destruction, more effective strategies will likely become possible. These may well involve combined approaches that add immunotherapy to conventional antitumor treatments or combine distinct immunotherapies attempting to obtain additive effects. For example, activation of the innate immune system with GM-CSF (neutrophils and macrophages) and IL-2 (NK cells) may have some antitumor activity, and is known to activate cells expressing Fc receptors. Infusions of tumor-reactive monoclonal antibodies can target the antibodies to sites of tumors *in vivo*, but tumor destruction may be limited by the ability of the antibody to use effector cells (cells with Fc receptors) to selectively destroy the tumor. Thus, combined treatment with cytokines such as GM-CSF and IL-2 together with tumor reactive monoclonal antibody [197] may allow these distinct mechanisms to work additively, allowing the activated effector cells to selectively recognize the tumor through *in vivo* ADCC. Ongoing progress with this and other forms of clinical cancer immunotherapy is presented in Chapter 14.

## ACKNOWLEDGMENTS

Research summarized in this chapter reflects experiences of various laboratory and clinical research teams worldwide, but this review does not attempt to cite comprehensively the massive body of literature in this expanding field. Work cited from our research teams are supported by National Institutes of Health (NIH)-NCI Intramural funds and NIH grants CA-32685, RR-03186, CM-87290, CA-53441, and CA-20432; NIH support to the University of Wisconsin General Clinical Research Center; GCRC; and the Midwest Athletes for Childhood Cancer Fund. Gratitude is given to our colleagues J. A. Hank and L. Helman, who have been involved in most aspects of these studies, and to editors Pizzo and Poplack for inviting us to provide this revised chapter.

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# 7

# CLINICAL ASSESSMENT AND DIFFERENTIAL DIAGNOSIS OF THE CHILD WITH SUSPECTED CANCER

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## SIGNS AND SYMPTOMS IN THE CHILD WITH CANCER

It is usually difficult to diagnose childhood cancer in its early stages because most of the signs and symptoms are relatively nonspecific and may mimic a variety of other, more common childhood disorders. For a pediatric oncologist, the index of suspicion of cancer is high; for the primary care physician, the opposite is true. The early manifestations are vague, childhood malignancies are rare (see Chapter 1), and there may be a reluctance to suggest such a diagnosis on the basis of indefinite signs and symptoms because of its ominous implications to the family. However, even though the patient or parents may not directly express it, they are often worried about whether an unexplained fever or lump represents a serious illness such as a malignancy. It is therefore appropriate that the possibility of cancer should be mentioned when the initial signs and symptoms are suspicious. The time to diagnosis of pediatric cancer is variable and ranges from a median time of 31 days for neuroblastoma to 136 days for Hodgkin's disease (Table 7-1).[1]

| Diagnosis | n | Mean | Median | 25th percentile | 75th percentile |
|---|---|---|---|---|---|
| Brain | 194 | 211 | 93 | 38 | 237 |
| Ewing's | 82 | 182 | 127 | 79 | 255 |
| Hodgkin's | 143 | 223 | 136 | 49 | 270 |
| Leukemia | 908 | 109 | 52 | 20 | 129 |
| Non-Hodgkin's lymphoma | 184 | 117 | 62 | 25 | 141 |
| Neuroblastoma | 237 | 120 | 58 | 15 | 164 |
| Osteosarcoma | 67 | 127 | 98 | 40 | 191 |
| Rhabdomyo-sarcoma | 126 | 127 | 55 | 25 | 161 |
| Wilms' | 223 | 101 | 31 | 9 | 120 |

**TABLE 7-1. DISTRIBUTION OF LAG TIME IN DAYS BY DIAGNOSIS OF COMMON CHILDHOOD CANCERS**

Obtaining the history is the first step in the diagnostic process, with the chief complaint being the most important initial clue. Some of the signs and symptoms that the public associates with the much more common adult-onset malignancies are rare in children. These include hoarseness, difficulty in swallowing, breast mass, chronic cough, a non-healing skin lesion, difficulty in voiding, bleeding from the rectum, and a change in bowel habits.

The signs and symptoms that are common to many childhood illnesses and possible malignancies are listed in Table 7-2. Initial evaluation for a malignant neoplasm is usually not warranted. If these signs or symptoms do not subside within a reasonable period, then a telephone consult with a pediatric oncologist or other specialist is warranted. There are exceptions to this generalization. For instance, a soft tissue mass on the torso or extremities in a child younger than 1 or 2 years of age warrants early evaluation and biopsy unless it is known that there was some traumatic event that could have caused such a bump.

| Symptom/sign | Possible malignancy |
|---|---|
| Generalized malaise, fever, adenopathy | Lymphoma, leukemia, Ewing's, NBL |
| Head and neck | |
| Headache, nausea, vomiting | Brain tumor, leukemia |
| Febrile seizure | Brain tumor |
| Earache | STS |
| Rhinitis | STS |
| Epistaxis | Leukemia |
| Pharyngitis | STS |
| Adenopathy | NBL, thyroid, STS, lymphoma, leukemia |
| Thorax | |
| Extrathoracic | |
| Soft tissue mass | STS, PNET |
| Bony mass | EWS, NBL |
| Intrathoracic | |
| Adenopathy | Lymphoma, leukemia |
| Abdomen | |
| External soft tissue | STS, PNET |
| Internal: diarrhea, vomiting, hepatomegaly, and/or splenomegaly | NBL, lymphoma, hepatic tumor, leukemia |
| Genitourinary | |
| Hematuria | Wilms', STS |
| Trouble voiding | Prostatic or bladder STS |
| Vaginitis | STS |
| Periscrotal mass | STS |
| Musculoskeletal | |
| Soft tissue mass(es) | RMS, other STS, PNET |
| Bony mass/pain | Osteosarcoma, EWS, NHL, NBL, leukemia |

EWS, Ewing's sarcoma; NBL, neuroblastoma; NHL, non-Hodgkin's lymphoma; PNET, primitive neuroectodermal tumor; RMS, rhabdomyosarcoma; STS, soft tissue sarcoma, including rhabdomyosarcoma.

**TABLE 7-2. SYMPTOMS AND SIGNS OF CHILDHOOD CANCER MIMICKING NORMAL CHILDHOOD ILLNESSES; INITIAL EVALUATION FOR CANCER USUALLY NOT WARRANTED**

There are signs and symptoms that are cause for alarm and concern (Table 7-3) and that warrant immediate evaluation to establish the diagnosis. If at all possible, these children and their families should be referred to a pediatric cancer center. There are multiple reasons for this. In addition to evaluation of the physiologic status of the child, the extent of disease should be evaluated by appropriate laboratory and imaging studies. In addition to a pediatric oncologist and surgical specialist, all of the other disciplines that may be involved in the initial evaluation, diagnosis, and subsequent management should be consulted. For instance, if the lesion is located on an extremity, a radiation oncologist should be consulted to make sure that subsequent radiation therapy (if required) will not be compromised by an inappropriately placed incision.

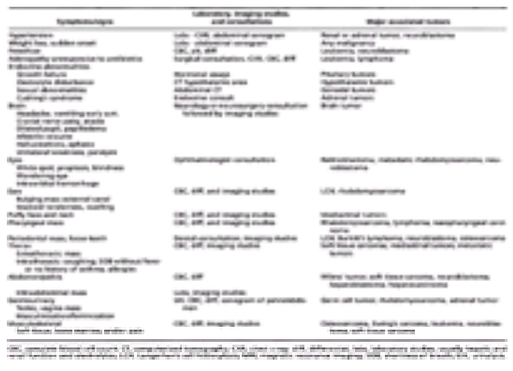

**TABLE 7-3. UNUSUAL SYMPTOMS AND SIGNS THAT WARRANT IMMEDIATE LABORATORY AND/OR IMAGING STUDIES AND CONSULTATION**

A pathology consult is absolutely essential. He or she will make sure at the time of surgery that enough appropriate material is obtained to establish the diagnosis. Studies will include not only routine histochemical and immunochemical stains but also immunotyping, cytogenetics, molecular genetics, electron microscopy, and, when warranted, anaerobic, aerobic, fungal, and other cultures for fastidious organisms. If the pathologist can confirm while the child is still asleep that the lesion is malignant, other suspicious lesions can be biopsied or excised. A bone marrow aspirate and lumbar puncture can be performed and a central venous access inserted.

At least 85% of pediatric cancers are associated with the presenting signs and symptoms listed in Table 7-2 and Table 7-3. The remaining 10% to 15% of tumors are those associated with unusual signs and symptoms that can make early diagnosis even more difficult. The pediatric tumor most commonly associated with unusual signs and symptoms is neuroblastoma (Table 7-4). Table 7-5 lists unusual presentations that have been reported for other pediatric malignancies.

| |
|---|
| Unusual signs and symptoms not related directly to tumor growth |
| Chronic diarrhea[2] |
| Polymyoclonus-opsoclonus[3] |
| Cog-wheel erythrocytes[4] |
| Failure to thrive[5] |
| Cushing's syndrome[6] |
| Unusual signs and symptoms related directly to tumor growth |
| Horner's syndrome[7] |
| Superior vena cava syndrome[8] |
| Hydrocephalus |
| Meningeal involvement[9] |
| Cavernous or lateral sinus involvement[10] |
| Choroid papilloma[11] |
| Blindness[12] |
| Subcutaneous nodules[13] |
| Leukemoid reaction[14] |
| Myasthenia gravis[15] |
| Heterochromia[16] |

**TABLE 7-4. UNUSUAL PRESENTATIONS OF CHILDHOOD NEUROBLASTOMA**

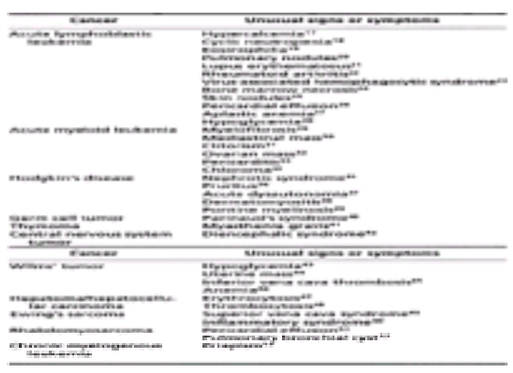

**TABLE 7-5. UNCOMMON PRESENTATIONS OF CHILDHOOD CANCERS OTHER THAN NEUROBLASTOMA**

The family medical history is of paramount importance when the diagnosis of malignancy is a possibility. Detailed histories of the parents, siblings, and first cousins should be noted on a family tree or family group record. The minimal data collected should include a list of serious illnesses, family members' ages, congenital anomalies, and the cause of death for deceased members. The occurrence of any malignancy should be documented with as much demographic data as are available on the diagnosis.

When dealing with pediatric tumors, genetic factors may be important. Established associations with genetic traits and other neoplasms or disorders are summarized elsewhere (see Chapter 3). The major categories of disease linked with an increased risk of cancer are immunodeficiency disorders, metabolic disorders, disorders of chromosomal instability, and the phakomatoses. Certain familial and genetic diseases, such as autoimmune diseases, neurofibromatosis, and familial polyposis are associated with an increased incidence of cancer.

Because outcomes for children with cancer have improved, there is a growing cohort of survivors of childhood cancers who have increased risk for development of a second malignancy.[54],[55] and [56] The association of osteosarcoma and bilateral retinoblastoma[57] and that of acute lymphocytic leukemia (ALL) and brain tumors[58] are good examples of such high-risk populations (see Chapter 2 and Chapter 49).

Environmental factors also are associated with increased risk for childhood cancer. Of the recognized oncogenic environmental factors, exposure to ionizing radiation[59] and oncogenic viruses (e.g., human immunodeficiency virus, Chapter 25, and possibly Epstein-Barr virus, Chapter 5) are the most commonly reported.

## DIFFERENTIAL DIAGNOSIS

The following sections discuss some of the more common signs and symptoms seen with pediatric cancer and suggest a method of workup for each one. Table 7-3 lists these signs and symptoms and the differential diagnoses and suggested diagnostic studies.

### Headaches

Headache is a common symptom seen in a pediatric practice. Although few of these headaches are caused by intracranial tumors, it is always important to rule out a brain tumor when dealing with complaints of repeated headaches (see Chapter 27).

The diagnosis of a brain tumor is initially suspected on the basis of a symptom complex that reflects the site of the tumor. Pediatric brain tumors are frequently situated so that they interfere with cerebrospinal fluid circulation, and increased intracranial pressure is a common occurrence. An analysis of the early prominent symptoms in a group of children with brain tumors revealed that with supratentorial tumors vomiting occurred in 46% of patients and headache in 43%, whereas with infratentorial tumors, 59% of patients had coordination difficulties, 76% experienced vomiting, and 56% complained of headache. [60]

Computed tomography (CT) and magnetic resonance (MR) with or without contrast imaging offer precise, noninvasive means to investigate the possibility of a brain tumor (see Chapter 9 and Chapter 27). However, these tests cannot be recommended routinely for all children with headache or vomiting. Honig and Charney [61] analyzed the history, physical examination findings, and skull radiographs of 72 children with headaches secondary to brain tumors. Their findings suggest that the best method of screening for a brain tumor in a patient with a headache is a careful neurologic examination, because approximately 95% of children with a headache and a brain tumor had abnormal neurologic findings on clinical examination. [61]

Obtaining a good clinical history is extremely important. The following variables should be determined: duration of symptoms and their location, timing, severity, precipitating events, and mode of onset. The study by Honig and Charney [61] indicated the importance of the following symptoms in suggesting a brain tumor: recurrent morning headache; headaches that awaken the child; intense and incapacitating headache; and changes in the quality, frequency, and pattern of the headaches. The conditions that suggest the need for further radiologic examination are outlined in Table 7-6. These examinations should be combined with a thorough neurologic examination.

| |
|---|
| Presence or onset of neurologic abnormality |
| Ocular findings such as papilledema, decreased visual acuity, or loss of vision |
| Vomiting that is persistent, increasing in frequency, or preceded by recurrent headaches |
| Change in character of headache, such as increased severity and frequency |
| Recurrent morning headaches or headaches that repeatedly awaken child from sleep |
| Short stature or deceleration of linear growth |
| Diabetes insipidus |
| Age of 3 yr or younger |
| Neurofibromatosis |
| Cured of acute lymphoblastic leukemia with irradiation of central nervous system as part of initial treatment |

**TABLE 7-6. CONDITIONS SUGGESTING THE NEED FOR COMPUTED TOMOGRAPHY IN CHILDREN WITH HEADACHE**

### Lymphadenopathy

Lymphadenopathy is a common complaint and physical finding in children. The size of the lymph nodes rapidly increase in size during the first 12 years of the child's life to such an extent that the total lymph node mass will be twice the size of that seen in an adult ( Fig. 7-1).[62] The size of the nodes in children vary markedly because the child is continuously exposed to new viruses and bacteria. The lymphocytes react to this exposure by markedly increasing their number to combat any invasion. A lymph node is considered enlarged if it is more than 10 mm in its greatest diameter. Exceptions are epitrochlear nodes, for which 5 mm is considered abnormal, and inguinal nodes, which are not considered abnormal unless they are larger than 15 mm. Most children have palpable, small cervical, axillary, and inguinal nodes, but adenopathy in the posterior auricular, epitrochlear, or supraclavicular area is definitely abnormal. Most enlarged nodes in children are related to infections.

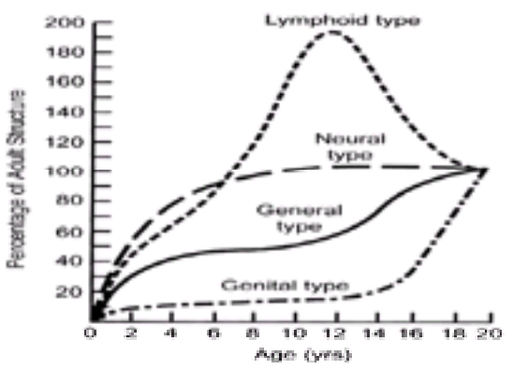


**FIGURE 7-1.** Growth of lymphoid tissue, neural cells, and supporting structures in the central nervous system; general somatic growth; and growth of secondary sex characteristics in a child from birth to adulthood. (Redrawn from Harris JA, et al: Measurement of man. University of Minnesota Press, 1930.)

A thorough physical examination and history are essential to the initial evaluation of lymphadenopathy. One of the first determinations to be made is whether the nodal enlargement is regional or generalized. Conventionally, lymphadenopathy is considered generalized when nodes are enlarged in two or more noncontiguous lymph node areas. It would be unusual to have generalized adenopathy without associated findings. Ordinarily, there is no problem in categorizing generalized lymphadenopathy fairly quickly after a thorough history and physical examination and a few radiographic and blood tests. For example, although ALL and acute myeloid leukemia (AML) often present with generalized lymphadenopathy, the enlarged nodes are usually not the sole presenting complaint and are only one of multiple abnormal clinical and laboratory findings. Although it is impossible to generalize enlarged lymph nodes secondary to malignant tumors, they are usually firm, rubbery, and matted. Cancerous nodes are usually not associated with tenderness, erythema, warmth, or fluctuance. Over the course of several examinations, an increase in nodal size is typically noticed by the examiner in malignant conditions.

Regional lymphadenopathy that predominates in noncervical areas is more suggestive of a malignancy than is node enlargement in the area of the head and neck. Any asymptomatic node larger than 2.5 cm merits further investigation and early consideration for excisional biopsy. Non-Hodgkin's lymphoma and neuroblastoma are two of the more common malignant conditions that manifest with regional lymphadenopathy. Even though the nodal enlargement is localized, there is often other evidence of systemic disease, such as a chest mass, an abdominal mass, or peripheral blood changes.

The differential diagnosis of localized node enlargement in the head and neck area is more difficult because acute and chronic infections are frequent causes of node enlargement. The most common bacterial causes of acute cervical adenopathy are *Staphylococcus aureus* and b-hemolytic *Streptococcus*.[63] Acute bacterial adenitis is usually associated with local signs of inflammation. Other infectious causes of cervical node enlargement include cat-scratch disease, nontuberculous mycobacterial infection, toxoplasmosis, infections with Epstein-Barr virus, cytomegalovirus, or human immunodeficiency virus. Isolated enlargement of the supraclavicular nodes usually signifies diseases transported by the thoracic ducts arising in the abdomen (Virchow's node) if on the left side, or from the chest if on the right.

With chronic, persistent, or progressive lymphadenopathy in the head and neck, malignancy becomes a more likely diagnosis. The primary malignant tumors that involve the head and neck in children are most often lymphomas. The child's age offers important diagnostic information and direction in evaluating enlarged nodes of the head and neck region for possible cancer. In children younger than 6 years of age, the most common cancers with head and neck involvement are neuroblastoma, rhabdomyosarcoma, non-Hodgkin's lymphoma, and leukemia. From 7 to 13 years of age, Hodgkin's lymphoma and lymphosarcoma are the most common and are seen with equal frequency. Thyroid cancer and then rhabdomyosarcoma follow in frequency. Over 13 years of age, Hodgkin's disease is the most frequent neoplasm.

Lymphadenopathy in the mediastinum presents a diagnostic problem. In a report by Bower and co-workers of 173 cases of mediastinal masses, 41% were malignant neoplasms.[64] At the University of Minnesota, of 68 anterior or middle mediastinal masses, 43% proved to be Hodgkin's lymphoma, 25% were non-Hodgkin's lymphoma, 17% were leukemia, and 9% were histoplasmosis.[65]

The diagnostic workup for lymphadenopathy should include the important clues from the clinical presentation as a guide. In general, if a lymph node is unresponsive to antibiotics and continues to increase in size over a 2-week period, it should be biopsied. Children with mild to moderately enlarged nodes at the first visit can be followed by serial examinations. Within 2 or 3 weeks, most noncancerous nodes should have regressed toward normal size. For localized cervical adenitis with

moderate inflammation and fever, the child should be started on oral antibiotic therapy empirically. The antibiotic should cover *Streptococcus* and *Staphylococcus*, and anaerobic coverage should be considered if a dental source is suspected. [63] A complete blood cell count (CBC) and a throat culture are indicated in these situations.

When a child does not respond to antibiotic therapy or has a fluctuant lymph node or a node larger than 2.5 to 3.0 cm, a more extensive workup is indicated. This may include a tuberculin purified protein derivative skin test, chest radiograph, CBC with differential and platelet count, and cultures from other sites that may be the source of an infection. Viral, bacterial, and fungal serologies are useful only if indicated by the history and physical examination. A bone marrow biopsy and aspiration are indicated if the chest film is abnormal, if the blood work shows thrombocytopenia or anemia, if there is significant hepatosplenomegaly, or if there is a combination of these parameters.

The need for lymph node biopsy is suggested by the following signs and symptoms:

- An enlarging node or nodes that remain enlarged after 2 to 3 weeks of antibiotic therapy
- Nodes that are not enlarging but that have not diminished in size after 5 to 6 weeks, or that do not return to normal size by 10 to 12 weeks, especially if associated with unexplained fever, weight loss, or hepatosplenomegaly (enlarged supraclavicular or lower neck nodes should be biopsied earlier)
- Enlarged nodes associated with any abnormal chest film finding

The largest and firmest node should be taken for evaluation. As mentioned previously, before this procedure is done, the child should be referred to a pediatric cancer center. If this is not possible, a pediatric oncologist and pathologist should be consulted by telephone. When suspect, material should be cultured for aerobic and anaerobic bacteria and for mycobacteria. In endemic regions, fungal cultures (e.g., for *Histoplasma* or *Cryptococcus*) should be done. The node should also be examined by light and electron microscopy and portions should be sent for chromosomal analysis and for analysis of cell markers. When possible, extra material should be frozen to have available if other tests become necessary.

**Thoracic Masses**

Almost all of the primary intrathoracic neoplasms develop within the mediastinum. Anatomically, the mediastinum is divided into three regions, and the localization of a mass in one of these compartments imparts important clues about its nature. The anterior mediastinum is bounded by the first rib superiorly, by the posterior surface of the sternum anteriorly, by the anterior border of the upper dorsal vertebra posteriorly, and by a curved line along the anterior cardiac border, extending back to the border of the dorsal vertebra. The posterior mediastinum is bounded posteriorly by the anterior surface of the curve of the ribs, anteriorly by the posterior border of the pericardium, and inferiorly by the diaphragm. The middle mediastinum occupies the area between the other two regions, with its base on the diaphragm.

The common lesions in the anterior mediastinum are lymphomas, masses of thymic origin, teratomas, angiomas, lipomas, and thyroid tumors. In the middle mediastinum, lymphomas, metastatic or infection-related lesions involving lymph nodes, direct extension of abdominal malignant lesions, pericardial cysts, bronchogenic cysts, esophageal lesions, and hernias through the foramen of Morgagni are found. In the posterior mediastinum, neurogenic tumors are some of the most common tumors, accounting for approximately 20% of all primary mediastinal tumors and cysts.[66] These lesions arise from the nerve roots and sympathetic ganglia in the paravertebral sulcus. The types seen are neuroblastomas, ganglioneuroblastomas, neurilemomas, neurofibromas, ganglioneuromas, and pheochromocytomas (rare). The other masses seen in the posterior mediastinum include enterogenous cysts, thoracic meningocele, and malignant tumors such as Ewing's sarcoma, lymphoma, and rhabdomyosarcoma.

Patients with mediastinal tumors may be asymptomatic or may present with symptoms secondary to compression or erosion of adjacent organs such as the respiratory tract (e.g., cough, stridor, and hemoptysis; see Chapter 39). Commonly, the mass is discovered during routine chest radiographs. Tomography, barium swallow, fluoroscopy, ultrasound, CT, and MR scans may be helpful in localizing and identifying mediastinal tumors (see Chapter 9). In all cases, the final diagnosis must await histologic definition.

A bone marrow aspiration and biopsy are frequently indicated for evaluation of possible tumor or infection. If marrow test results are negative, more invasive diagnostic procedures, such as bronchoscopy, mediastinotomy, or open biopsy, are necessary to obtain tissue for histologic study.

A common diagnosis made by analysis of tissue from the mediastinum is that of a small round cell tumor (see Chapter 8). This category includes Ewing's tumor of bone and soft tissue, rhabdomyosarcoma, lymphoma, and leukemic mass. Electron microscopy, histochemical evaluation, cell membrane studies, cytogenetics, and polymerase chain reaction studies may be necessary to establish a definitive diagnosis. If one is in doubt, and a portion of a rib is involved, resection of the rib above and below the lesion, as well as resection of the involved rib and intercostal muscle along with the tumor, is justified. This procedure satisfies the criteria for initial treatment of Ewing's tumor, rhabdomyosarcoma, and other small blue round cell tumors of thoracopulmonary origin (see Chapter 31, Chapter 32, Chapter 33, Chapter 34 and Chapter 35).

**Bone and Joint Pain**

Most pain associated with cancer is caused by bone, nerve, or visceral involvement or encroachment (see Chapter 43). The early symptoms of childhood cancer rarely include pain, except for malignancies involving bone or bone marrow, such as leukemia and primary bone cancers, or metastatic diseases to bone or bone marrow (e.g., neuroblastoma). Bone pain is common for the two most common malignant bone tumors in children, osteogenic sarcoma and Ewing's tumor. In a series of 229 patients with Ewing's tumor, 89% had pain as a presenting symptom (see Chapter 33 and Chapter 35).[67] This pain tends to be intermittent at first, and increases in severity with time. A peculiarity of the pain associated with Ewing's tumor is that it often disappears spontaneously for weeks or months. A similar incidence of bone pain is seen with osteogenic sarcoma, with pain as a presenting symptom for 79% of the cases. [68] For osteogenic sarcoma and Ewing's tumor, the median time between the onset of symptoms and the diagnosis was 3 months and 4 months, respectively (see Table 7-1).

Bone pain or arthralgias may be prominent presenting features of acute leukemia, and leukemic joint pain can be mistaken for various rheumatic diseases. For a child with suspected arthritis, the presence of leukopenia or nondiagnostic bone scan should prompt the physician to examine the bone marrow (see Chapter 19). Clinical findings and constitutional complaints do not help to differentiate the causes of bone pain. Bone pain as a presenting complaint of leukemia has been reported in 27% to 33% of cases.[69] Most of the cases are related to ALL rather than to acute nonlymphocytic leukemia. A review of 107 consecutive children presenting with ALL reported 21% with bone pain and 44% with radiographic bone changes. [70] A significant correlation was found between the severity of the bone pain and the number of bones demonstrating radiographic involvement. There was no significant correlation between the presence or absence of bone pain and the prognosis.

If a child complains of persistent bone or joint pain, especially if the pain is abrupt in onset, occurs at night, and is associated with swelling, mass, or limitation of motion, a radiograph should be obtained promptly and examined by an experienced radiologist. Although the films are extremely important in judging the extent of the abnormality and the behavior of the skeletal lesion, they should not be used to make a definitive diagnosis of the cause, because there are no pathognomonic radiographic signs of malignant bone lesions. Biopsy and pathologic studies are absolutely necessary to establish the diagnosis.

**Abdominal Masses**

A palpable abdominal mass is one of the most common presenting finding of malignant solid tumors in children. Although there are a variety of benign or pseudotumorous entities that may manifest as abdominal masses, all masses require workup to ensure that an early proper diagnosis is made.

In obtaining the history, it helps to determine whether symptoms have been referable to the abdominal mass and, if so, their type and duration. Because of the high incidence of renal cause for an abdominal mass, a thorough history that focuses on the urinary tract is particularly important.

The age of the patient is a helpful clue. For an abdominal mass in a newborn infant, a renal cause is most common. If the mass turns out to be malignant, it will most likely be a Wilms' tumor or a neuroblastoma ( Table 7-7). In older children, a mass is more likely to be secondary to leukemic or lymphomatous involvement of the liver and spleen.

| Retroperitoneal tumors (462 cases) | Number of patients |
|---|---|
| Kidney | |
| Wilms' tumor | 202 |
| Other malignant tumors | 10 |
| Benign tumors | 18 |
| Sympathetic tumors | |
| Neuroblastomas with metastases at presentation | 125 |
| Neuroblastomas without metastases | 60 |
| Neuroblastomas (Pepper's syndrome)[a] | 15 |
| Ganglioneuromas | 5 |
| Adrenocortical tumors | 7 |
| Malignant lymphomas | 65 |
| Liver | |
| Benign tumors | 9 |
| Malignant tumors | 14 |
| Ovary | |
| Dysgerminomas | 4 |
| Teratomas | 27 |
| Embryonic sarcomas | 22 |
| Other | 9 |

Data from the Institut Gustave-Roussy as presented in Shweisguth O, ed. Solid tumors in children. New York: John Wiley & Sons, 1982, with permission.
[a]"Pepper's syndrome" eponym for neuroblastoma of adrenal gland (usually right) with hepatic metastases.

**TABLE 7-7. RELATIVE INCIDENCE OF RETROPERITONEAL TUMORS**

For the child with a mass, a thorough abdominal examination is frequently not easy. Every attempt should be made to have the child relaxed before palpating the abdomen. Attempting to divert the child's attention is perhaps the best method but does not always work. For a younger child, a bottle or pacifier can be helpful. When examining the abdomen, it is important to remember that a number of structures (i.e., liver edge, spleen, kidneys, aorta, sigmoid colon, feces, and/or spine) are palpable in normal children. Further studies depend on whether the child has a normal CBC. If the abdominal mass is suspected of being feces, it may be practical to give an enema and reexamine the patient. Similarly, if the mass is suspected to be the bladder, it may be necessary to catheterize the patient and repeat the examination. A rectal examination is indicated if the child has a normal absolute neutrophile count. Vaginal and pelvic examinations are important in the older adolescent girl, but bimanual abdominal and rectal examinations are preferred to vaginal examinations in infants and younger girls. Pelvic examinations should be performed by an experienced physician. Although much has been written about the importance of the size, mobility, and consistency of a mass, such information is unrewarding in determining its nature. Histologic confirmation is always necessary.

After the physical examination has been completed and routine laboratory studies obtained, the workup should proceed to sonography of the abdomen and a chest film. The results may suggest the need for any further tests such as abdominal CT with or without contrast and intravenous urography, tumor marker assays, and bone marrow examination.

## Pancytopenia and Leukocytosis

### *Pancytopenia*

Anemia, leukopenia, and thrombocytopenia occur alone or in combination as a common presenting sign in acute leukemias of childhood, both ALL and AML (see Chapter 19 and Chapter 20). Among 936 untreated children with newly diagnosed ALL, 51% presented with a hemoglobin concentration of less than 7.5 g per dL, 73% presented with platelet counts less than 150,000 per $mm^3$, and 30% presented with a total peripheral white blood cell count (WBC) of less than 5,000 per $mm^3$.[71] A similar analysis of 171 children with AML revealed that 82% presented with platelet counts less than 100,000 per $mm^3$, and 39% presented with a total WBC of less than 5,000 per $mm^3$.[72]

Any malignancy that involves the marrow can produce pancytopenia or depression of only one of the cell lines. After leukemia, the childhood malignancies that most often involve the bone marrow are neuroblastoma, lymphoma, and, less commonly, Ewing's tumor and rhabdomyosarcoma. These cytopenias are primarily attributed to replacement of the bone marrow by tumor, although such replacement often does not completely explain the degree of pancytopenia. There are alternative explanations for anemia associated with various malignancies. The anemia may be typical of that seen in chronic disease. Autoimmune hemolytic anemias may occur with lymphoma.

Unless there is marrow involvement, leukopenia is rarely a part of primary extramedullary malignancies. This is also true of thrombocytopenia, except for the rare instance of immune-mediated thrombocytopenic purpura associated with Hodgkin's disease, or the low platelet counts seen with a disseminated intravascular coagulopathy (DIC), which may occur with tumors such as neuroblastoma that have extensive tumor necrosis.

### *Leukocytosis*

An elevated WBC (i.e., leukocytosis) is commonly seen with acute leukemia of childhood (see Chapter 19 and Chapter 20). Counts of greater than 10,000 per $mm^3$ are reported in 45% of children with ALL; 10% have an initial count of greater than 100,000 per $mm^3$.[71] Among newly diagnosed cases of AML, 21% of patients have WBCs of greater than 100,000 per $mm^3$.[72] Most nonmalignant cases of leukocytosis are caused by infections, especially staphylococcal and pneumococcal infections. Exaggerated elevations (e.g., leukemoid reactions) of greater than 50,000 per $mm^3$ may occur with septicemia, especially with infections due to *Staphylococcus*, *Haemophilus influenzae*, *Meningococcus*, and *Salmonella*.[73] Lymphoid leukemoid reactions have been observed in infectious lymphocytosis,[74] mumps,[75] varicella,[76] adenovirus,[77] cytomegalovirus,[78] and pertussis[79] infections. Peripheral WBCs greater than 100,000 per $mm^3$ almost always reflect a leukemia. A myeloid leukemoid reaction (100,000 per $mm^3$) has been reported in premature infants whose mothers received corticosteroids during pregnancy.[80] Leukocytosis, predominantly due to eosinophilia, with WBCs in the range of 20,000 to 100,000 per $mm^3$, is often seen with parasitic infections, especially visceral larval migrans.[81] Other non-malignant causes of eosinophilia include the hypereosinophilic syndrome, periarteritis nodosa, allergy, and hypersensitivity reactions. The malignant causes are Hodgkin's disease, ALL, and a rare entity described as eosinophilic leukemia (see Chapter 19 and Chapter 20).

### *Workup of Pancytopenia and Leukocytosis*

Careful assessment for an infectious cause is of paramount importance in evaluating a patient with abnormal peripheral blood counts. A bone marrow study is frequently the most appropriate means to rule out most malignant causes, and aspiration and biopsy are required to determine morphology and marrow cellularity.

The indications for bone marrow examination include

- Finding of atypical or blast cells on peripheral blood smears
- Significant depression of more than one peripheral blood cell element without obvious explanation
- Association with unexplained lymphadenopathy or hepatosplenomegaly, or a thymic mass
- Absence of an infectious cause for the blood abnormality

## Bleeding

Bleeding is an uncommon initial sign in children with cancer. When it does occur, it is usually related to thrombocytopenia secondary to marrow involvement. In a patient with newly diagnosed acute leukemia, bleeding can also be related to impaired platelet function due to the administration of aspirin or other nonsteroidal antiinflammatory drugs for fever or bone pain. High-dose penicillin, carbenicillin, ticarcillin, and moxalactam have been associated with platelet dysfunction and can add to the bleeding tendency of these patients.[82] Effective antibiotics without these side effects are available (see Chapter 41). Although coagulation abnormalities can be associated with disseminated malignancies, they rarely cause signs or symptoms unless DIC occurs. A very high incidence of coagulopathy occurs in cases of acute promyelocytic leukemia (M3) and to a lesser extent with myelomonocytic leukemia (M4) and acute monoblastic leukemia (M5a) when associated with initial leukocytosis.[83,84] This complication has also been reported for patients with ALL (especially T-cell ALL), lymphoma, and neuroblastoma.[85] When a child presents with significant bleeding and thrombocytopenia, the possibility of a malignancy, especially acute leukemia, and other causes, such as infection and immune-mediated thrombocytopenia, must be considered. The diagnosis usually requires examination of the peripheral blood with a CBC and frequently necessitates a bone marrow aspiration. Initial laboratory workup of a bleeding diathesis consists of a standard panel of tests: one-stage prothrombin time (PT), activated partial thromboplastin time (PTT), thrombin time, fibrinogen level, serum fibrin-fibrinogen degradation (split products), and platelet count. In a patient with DIC, the PT is prolonged, and the PTT usually prolonged; the thrombin time is slightly prolonged, and fibrinogen is decreased, with decreased platelet count and increased fibrin degradation products. The presence of fibrin degradation products usually separates DIC from other causes of bleeding that are associated with abnormal PT, PTT, and fibrinogen levels.

Only rarely is the bleeding associated with malignancy difficult to detect clinically. Consultation with coagulation experts assists in selecting more specialized studies.

## ESTABLISHING THE DIAGNOSIS

With the exception of the rare true emergency, optimal treatment for a malignancy can begin only after the tumor has been accurately diagnosed and the extent of disease precisely defined. Noninvasive imaging techniques, such as CT, diagnostic ultrasound, and MR imaging, have dramatically improved the assessment for cancer (see Chapter 9). Significant advances have been made in identifying cellular tumor markers. Although extra-cellular markers, such as the catecholamines and alpha-fetoprotein, have direct clinical usage, many have no direct clinical application, and, whereas they potentially provide important clues to the presence of a cancer, the only absolute way to establish the diagnosis of cancer is by pathologic confirmation.

When initiating the evaluation of a child for a suspected cancer, it is important to discuss overall plans with the parents and the child (if of appropriate age). Most families, and often referring physicians, are frustrated with the length of time that the diagnostic tests, including biopsy and interpretation, take. For example, routine processing and examining a tissue specimen usually take 2 or 3 days but may take longer if special techniques or preparations are needed to make a definitive diagnosis. The family's frustration and anxiety are heightened when the signs and symptoms have persisted for months. It helps to prospectively emphasize to the parents that enough time must be taken to ensure the correct diagnosis and to determine the extent of disease accurately. Such care helps ensure proper therapy and prevents later errors and the need to repeat examinations and biopsies after treatment has begun.

### Pathologic Diagnosis

When the results of the clinical, laboratory, and imaging studies point to the probability of a neoplasm, the next decision is selection of the quickest and most reliable method to establish the pathologic diagnosis (see Chapter 8). Before obtaining any tissue for pathologic study, the primary physician should confer with a pediatric oncologist, the surgeon, and the pathologist and discuss the site to biopsy, the amount of tissue needed, and the specimens to be obtained. It is important to plan to remove enough material initially so that additional biopsies are not necessary.

The basic principles of initial surgery for childhood tumors are outlined in Chapter 12. In general, when it appears that the mass is localized to an organ such as the adrenal gland or the kidney, and there is no evidence of metastatic disease, the approach is one of surgical exploration, with the intent of total extirpation if possible. Although an intraoperative frozen-section study may be unnecessary because an immediate diagnosis is unlikely to alter the operative management, review of the surgical margins may help determine the adequacy of the excision (e.g., in cases of a partial hepatectomy for hepatoblastoma or resection of a soft tissue sarcoma). Additionally, if the lesion is malignant, central venous access and bone marrow aspirate or biopsy can be done while the child is still asleep.

Excisional and incisional biopsies are the standard techniques for obtaining diagnostic tissue. An excisional biopsy is preferred by most pathologists, because it yields a greater amount of tissue with fewer artifactual distortions than the smaller incisional biopsy. However, if an excisional biopsy could compromise future therapy (such as an extremity lesion, which may require radiation therapy), it should not be performed. The prototypical clinical situation for an excisional biopsy is the suspicious lymph node. Too often, an accessible lymph node is removed in fragments rather than as an intact structure. If a suspicious lesion is smaller than 4 cm and located superficially in the dermis or subcutaneous tissues, an excision generally is recommended. A larger mass in a deeper location may require a more cautious approach with an incisional biopsy unless a total resection is contemplated.

Frozen-section examination is recommended during an incisional biopsy to be certain the targeted lesion has been sampled. However, if the amount of available tissue is small or if the surgeon is reluctant to remove additional tissue because of a difficult approach, a frozen section should not be performed. Disadvantages of the frozen section include artifact, exhaustion of tissue in the block through sectioning, and the loss of the opportunity to perform additional studies such as electron microscopy. On the other hand, frozen section of tissue that is subsequently fixed in formalin generally does not interfere with the application of most immunohistochemical stains.

Regardless of the particular biopsy technique, the resultant tissue should be placed in 0.9% saline and immediately transported on ice to the surgical pathology laboratory. This method ensures the most expeditious handling of the tissue for special studies such as cell markers, cytogenetics, freezing for a variety of analyses, preparation of a suspension for flow cytometry, and fixation for electron microscopy. A pathologist should be available to supervise these activities. Gross examination of the tissue is preferred beforehand so that a judgment can be made about the representative nature and adequacy of the specimen.

Aspiration, biopsy, and cytology (ABC) using needle aspirate and biopsy or a fine needle biopsy (FNB) have been increasingly recommended in some clinical situations if small amounts of tissue can suffice for the intended purpose. Because of limitations of sample size, the ABC/FNB approach as the primary diagnostic procedure does not yield sufficient material for the myriad special studies that most pediatric oncologists prefer for these tumors before initiating therapy.

However, the approach has generated much enthusiasm by virtue of its cost efficiency, low morbidity, and reliability in experienced hands. The procedure has been widely applied in adults for thyroid, prostate, or breast masses. The method also can be used for the more deeply situated mass, with the thin-needle aspirations guided by CT scan. The skill of the operator and the confidence of the cytopathologist are the critical elements for a reliable diagnosis. An ideal clinical indication for ABC/FNB is the child with a previously diagnosed and treated neoplasm who presents with a suspected recurrence. [86] The tumor type will have already been established by an earlier biopsy or excision, and histopathologic confirmation is not a consideration unless there is a question of a second primary tumor. Even then, if the histologic types of the two primary tumors are sufficiently divergent, this may be appreciated in the ABC/FNB. The recognition of a recurrent neuroblastoma or rhabdomyosarcoma by ABC is usually straightforward.

Electron microscopy, immunohistochemistry, immunophenotyping of suspected lymphomas, cytogenetic DNA analysis through flow cytometry, and oncogene amplification studies are specialized diagnostic procedures available in many laboratories (see Chapter 8). Few laboratories have all of these techniques immediately available. The point cannot be overemphasized that these studies must be prefaced by an accurate pathologic diagnosis or reasonable differential diagnosis. The pathologist is in the best position in most cases to decide the appropriate special studies in consultation with the clinician.

## SUMMARY

Throughout this chapter, the emphasis has been on the influence of presenting signs and symptoms in making the early diagnosis of cancer in children. As shown in Table 7-8, the impact of age and site is important and should be considered when evaluating a sick child. The armamentarium of diagnostic tests available to establish the diagnosis of malignant diseases is extensive. Early diagnosis can improve outcome, but if the possibility of a cancer is never considered by the physician, delayed diagnosis is the result. Although the incidence of malignant disease in children is low, the impact of cancer makes it imperative that all professionals handling children have a high index of suspicion of cancer.

**TABLE 7-8. PREDOMINANT PEDIATRIC MALIGNANT TUMORS BY AGE AND SITE**

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# 8

# MOLECULAR PATHOLOGY OF PEDIATRIC MALIGNANCIES

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## OVERVIEW

Cancer in children is distinct from cancer in adults and requires a far different approach to diagnosis and treatment. The mere fact that most childhood tumors are mesenchymal or neuroectodermal in origin, whereas the overwhelming majority of adult cancers are of epithelial origin, clearly sets childhood cancer apart. [1,2,3,4 and 5] Furthermore, the primary treatment modality in adult cancer is surgery; in children, success was historically achieved through chemotherapy in combination with surgery and radiation therapy. The evolution of combination therapy protocols has brought with it a very real need to tailor treatment to specific types and subgroups of tumors in children, far more so than in adults. This demands far more precision and flexibility of the pediatric diagnostician. Finally, the highly organized character of childhood cancer treatment, whereby the vast majority (i.e., 90% or more of children aged 15 years and younger) is treated on cooperative group protocols, has mandated tumor-system–specific specialized diagnostics in conjunction with routine pathologic evaluation. [6] This task has been complicated by the unusual number of tumors in children that lack morphologic evidence of differentiation, and therefore histogenesis, the usual necessary first criterion for accurate diagnosis. Historically referred to as the *small round, blue cell tumors*, the problem is now well addressed with a battery of molecular and immunochemical diagnostic procedures, but only if such methods are used.[7,8] Many of these specialized diagnostic procedures are largely unknown to pathologists more familiar with adult cancer patients, and are thus frequently overlooked in the diagnostic workup of pediatric cancer patients in largely adult settings.

The net effect of these considerations in the diagnosis of childhood cancer is a need for clear, explicit guidelines for the diagnostic workup of children with tumors, as well as detailed consideration of specific diagnostic modalities required in the differential diagnosis of certain childhood cancers. In addition, certain tumor groups present particular problems in either diagnosis (such as the morphologically undifferentiated tumors) or prognostic subgrouping [as in leukemia, brain tumors, lymphoma, neuroblastoma (NB), rhabdomyosarcoma (RMS), and certain nonrhabdomyogenic soft tissue sarcomas]. This chapter sets out a set of guidelines for optimizing the likelihood of correct, specific, and clinically useful diagnosis in tumor patients, followed by a discussion of tumor systems that represent particular diagnostic or management challenges to the pathologist, oncologist, or surgeon. We hope that this information will be useful not just for the pediatric pathologist but also for anyone charged with diagnostic or management responsibilities for the child with cancer.

### Unique Challenge of Childhood Cancer Diagnosis: Age, Ethnicity, and Genetic Factors

Carcinoma is unusual in children. Retinoblastoma is unknown in adults. [9] In fact, childhood cancer bears little resemblance to adult cancer, as illustrated by a consideration of childhood tumor types in Figure 8-1. Clearly the common carcinomas (e.g., breast, lung, colon, prostate) dominate any consideration of adult cancer but are all absent from childhood cancer. In contrast, mesodermally derived tumors (e.g., hematopoietic and sarcomas) and brain tumors dominate childhood cancer, which reflects fundamental differences in the etiology of these two age groups. Many tumors seen in children are virtually unknown in adults, although due to the overwhelming preponderance of carcinoma in adults, the frequent occurrence of childhood tumors such as Ewing's sarcoma, osteosarcoma, synoviosarcoma (SS), and RMS in young adults is often overlooked. These are the same tumors, and should be recognized and treated as such.

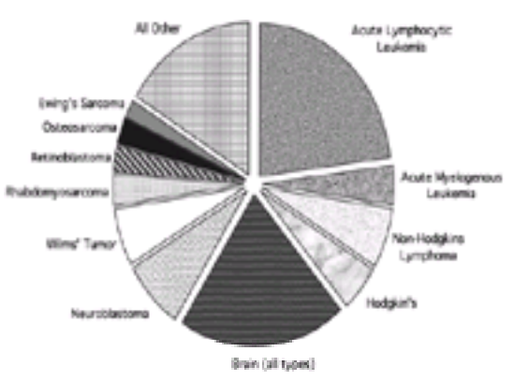


**FIGURE 8-1.** Distribution of childhood cancer. Hematopoietic malignancies (*gray textured wedges*) account for approximately one-third of childhood malignancy: acute lymphocytic leukemia and acute myeloid leukemia account for approximately 27%, and non-Hodgkin's lymphoma and Hodgkin's account for another approximately 11%. Brain tumors total approximately 21%. Neuroblastoma (approximately 7%) and Wilms' tumor (approximately 6%) follow. Rhabdomyosarcoma, retinoblastoma, osteosarcoma, and Ewing's sarcoma are each approximately 3% of the total. All other tumors account for approximately 16% of all childhood tumors in aggregate. Notably absent from the above is any significant incidence of carcinoma, the most common form of adult cancer.

#### *Age versus Tumor Type*

Beyond these obvious points lies a complex panoply of considerations that confront the diagnostician. It is difficult enough that many tumors defy simple morphologic

diagnosis. It is worse still that the occurrence of one or another type is quite age dependent, as illustrated in Figure 8-2, a graph of age versus tumor type from birth to 15 years. Overall, cancer incidence is greatest just after birth and declines to a low at age 10 years, reflecting intrauterine oncogenesis and growth for most of these tumors. However, there is also a linear increase after the first decade that extends into adulthood. This increase likely reflects the development of tumors with an etiology distinct from the largely embryonal tumors of early childhood yet still distinct from the common carcinomas of adulthood, in which environmental and lifestyle factors play a major role. No such factors are known for any childhood tumor.

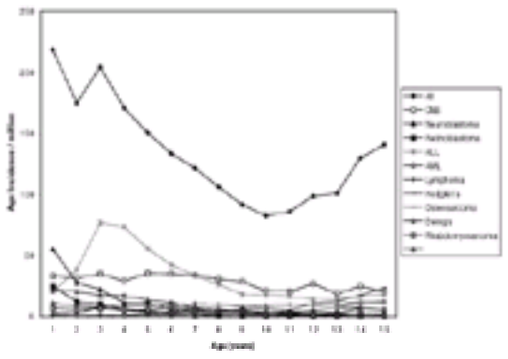

**FIGURE 8-2.** Age incidence of childhood cancer. All cancer as a group is depicted by the line with diamonds and shows a nearly linear decline from birth until approximately age 10 years, at which time the slope of the line reverses and begins to incline steadily toward greater incidence in adulthood. The large peak seen at approximately age 3 years is largely due to the peak incidence of acute lymphocytic leukemia at age 3 years ( ). The influence of other tumors is less apparent due to their lesser incidence, but here, too, striking age-dependent differences occur as well, especially with neuroblastoma ( ) and retinoblastoma ( ). ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CNS, central nervous system.

The issues are in fact even more complicated. Specific tumors show distinct age incidence within childhood. Figure 8-3 illustrates this in connection with common neuroectodermal tumors such as NB and the Ewing's family of tumors (EFTs); they have completely different age ranges, yet the two tumors are almost, in some cases, morphologically indistinguishable. Without knowledge of the patient's age, otherwise avoidable misdiagnoses may occur.

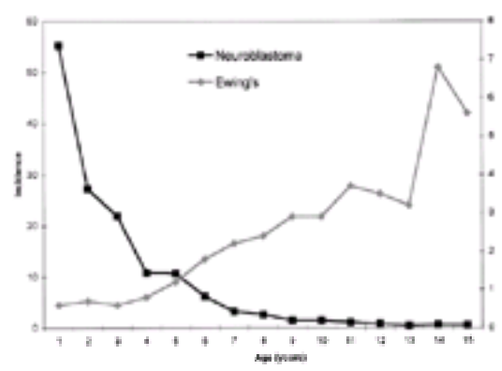


**FIGURE 8-3.** Neuroblastoma versus Ewing's sarcoma: age-specific incidence. Although the two most common neural tumors in childhood are neuroblastoma and Ewing's sarcoma, they are strikingly different in their incidence and character, despite their occasionally similar morphology. Neuroblastoma, however, is clearly a largely congenital tumor most common at birth, whereas Ewing's sarcoma is rare at birth and peaks at age 14 years. From this one would assume that the genetic origins for these two neural tumors are distinctly different (incidence = cases/million).

Even within a single tumor system, age plays a significant role. There are two dominant types of RMS, the most common sarcoma in children: embryonal (ERMS) and alveolar (ARMS). The age of occurrence between the two is quite different, however, as shown in Figure 8-4. ERMS, as the name suggests, is clearly linked to embryogenesis, with a peak incidence basically at birth, whereas ARMS shows a bimodal age incidence quite distinct from ERMS, such that in older patients, alveolar forms predominate, despite its overall lesser incidence (e.g., only one-half as common overall as ERMS). Not only does this distinction suggest a very different pathogenesis but it also complicates the diagnosis, as the two forms may appear morphologically similar (as discussed in section on classification of RMS). This has historically led to an approximate 20% misdiagnosis rate, based on cooperative group studies of this tumor. Misdiagnosis in turn has led to suboptimal treatment of many patients, as these same cooperative group treatment protocols distinguish between the two for treatment purposes.

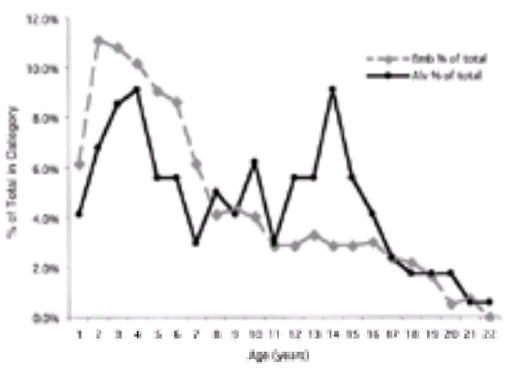


**FIGURE 8-4.** Age incidence of rhabdomyosarcoma. The two major forms of childhood rhabdomyosarcoma, embryonal (Emb) and alveolar (Alv), show distinctly different age incidences. Embryonal is most common in the first 2 years of life and declines steadily thereafter. Alveolar rhabdomyosarcoma, when plotted as a percent of total cases, shows a distinct bimodal age incidence, the first mode roughly corresponding to the peak age incidence of embryonal rhabdomyosarcoma, and the second at age 14 years, unlike embryonal forms. Recent studies indicate that the two modes are genetically and prognostically distinct as well.

### *Genetic Factors*

Age is an important consideration in the diagnostic evaluation of a patient, but it is also known that genetic factors, such as inherited gene defects, are not uncommon in childhood tumors; familial versus sporadic retinoblastoma is the prototypical example. [10] Familial patients with a mutation in one allele of the *RB* gene develop bilateral and multiple tumors when the normal allele is lost or mutated; sporadic patients generally do not. Li-Fraumeni syndrome, with a germline mutation of *p53,* is another, more pleiotropic example in which multiple tumor types throughout life can occur, presumably depending on when the normal *p53* allele is lost or mutated.[11,12]

In other cases, ethnicity can likewise be a critical determinant of cancer susceptibility. Here there is no classic mendelian gene defect as seen in retinoblastoma and Li-Fraumeni syndrome. Rather, as yet unknown, complex genetic traits are presumably responsible. The classic example of this is the EFTs. As shown in Figure 8-5, based on Surveillance, Epidemiology, and End Results network data, this tumor is virtually unknown in some African populations and is nearly as scarce in some Asian populations (e.g., in China and Hong Kong), whereas in northern Europeans it is more common than the “most” common bone sarcoma, osteosarcoma. [2,13,14]

Even more surprising, the tumor is most common in Spanish, Israeli (non-Arabic), and notably, Polynesian (e.g., Maori, Hawaiian) populations. Epidemiologic studies have documented other, less striking but nonetheless important differences in tumor type incidence among ethnic populations. [5,15] Aside from the intriguing implications for population-specific disease susceptibility, this observation implies that knowledge of patient ethnicity can be a helpful adjunct in diagnosis, particularly among the undifferentiated tumors common in childhood.

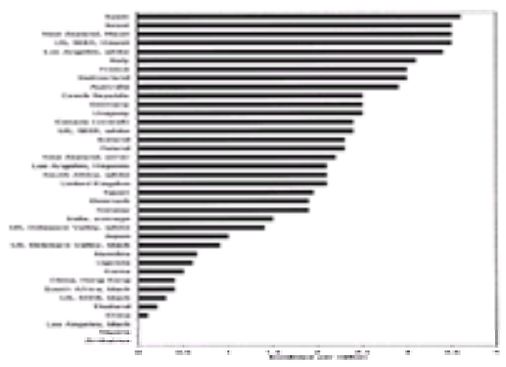

**FIGURE 8-5.** Ethnicity versus incidence—the Ewing's family of tumors. Striking differences in the incidence of Ewing's sarcoma are well documented and are graphically evident in this illustration. The well-known near-total lack of incidence in native Africans (Zimbabwe and Nigeria with no reported incidence) as opposed to a striking incidence in whites (Los Angeles, white, 3.4) fails to convey the entire story. Surveillance, Epidemiology, and End Results (SEER) network data clearly document an even greater incidence in Spain and Israel (approximately 3.5) as well as in at least two Polynesian populations, the New Zealand Maori and the Hawaiian Polynesian, with a similar incidence. At the same time, considerable variation among Asian populations is evident: China has the lowest reported incidence (0.1), Korea is intermediate (0.5), and Japan is the highest (1.0). These striking ethnic differences are not paralleled by any childhood malignancy.

## Current Approach to Childhood Tumor Diagnosis

Similar to all areas of medicine, pathology has been transformed by a rash of new techniques, equipment, and basic knowledge eventuating from the tremendous advances in biotechnology. This, in turn, has irrevocably changed the methods used to arrive at a diagnosis. No longer is gross examination and light microscopy in conjunction with clinical information the only means available to derive a diagnosis. Virtually every case of childhood cancer now requires recourse to some combination of immunochemical, molecular, or, occasionally, ultrastructural analysis. [16,17] and [18] These tools have enormously increased diagnostic precision, but most important, they allow far better correlation of tumor diagnosis with clinical behavior and response to therapy.

Examples of specific tumor types and their optimal diagnostic evaluation are discussed in the section Childhood Tumor Diagnosis. Here, mention of a few specific examples will suffice to make the point. Ideally, no child with suspected leukemia, lymphoma, or NB will fail to have ancillary diagnostic procedures performed to detect gene translocations, antigen expression, or gene amplification. This concentration of advanced diagnostic procedures common to childhood cancer evaluation is rare in adult cancer, a striking difference often overlooked by adult cancer diagnosticians. This is unfortunate, because these procedures require special handling considerations (e.g., fresh, viable tumor tissue, short-term culture, or fresh frozen tissue) that are not part of the routine workup of adult cancer. In many cases, local institutions lack the facilities to perform these studies, but the tissue is handled by prescribed protocols that ensure proper handling of tissue and shipment to a central reference laboratory to complete these studies. If these procedures are unknown to the primary pathologist, as they often are, the moment may be lost. Fresh tissue [and the high-quality, largely intact messenger RNA (mRNA) it contains] cannot be reconstituted from formalin-fixed tissue, nor can cell cultures for molecular genetic analysis or establishment of a permanent cell line be grown from fixed or frozen tissue. It is thus useful to lay out an action plan for childhood tumor diagnosis that will forestall such eventualities.

A simplified diagram, Figure 8-6, illustrates the routine handling of childhood cancer tissue as prescribed by the Children's Oncology Group, which is responsible for the diagnosis and treatment of as much as 95% of children with cancer in North America. [19] A sizable percentage of these cases is not handled by pediatric pathologists familiar with these protocols; inclusion here will hopefully assist the general pathologist confronted with childhood cancer cases. The diagram illustrates the single most important principle: *tissue must not be placed into fixative in the operating room*. This single oversight is responsible for most lost diagnostic opportunities. It is also a reason that so little tissue is available for molecular diagnostics and biomedical research. Fixed tissue has limited utility for most molecular genetic diagnostic procedures and severely limits even conventional diagnostics such as immunohistochemistry and electron microscopy (EM).

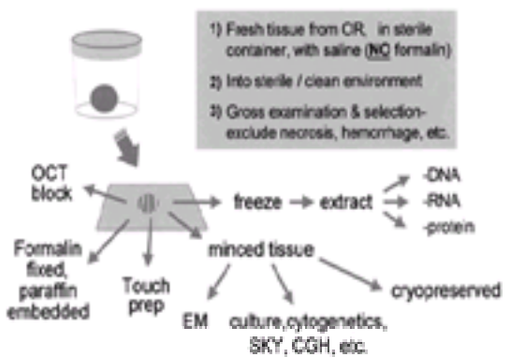


**FIGURE 8-6.** Tissue handling diagram. Tissue for optimal pathologic and biologic studies requires specialized handling, as summarized in this chart. Tissue should be collected fresh, not fixed. From a fresh or frozen specimen, virtually all important studies can be performed. Increasing reliance on specialized molecular diagnostics, especially those that detect messenger RNA, require fresh or fresh frozen tissue for routinely successful results. A version of this diagram is in wide use in the Children's Oncology Group and has contributed to significant improvements in diagnostic accuracy in childhood cancer. CGH, comparative genomic hybridization; EM, electron microscopy; OCT, optimal cryopreservation temperature compound; OR, operating room; SKY, spectral karyotyping.

The second stage of tissue handling illustrated in the diagram is simply precautionary: If tissue is divided into multiple forms, for specific use as needed in subsequent diagnostic procedures, nothing is lost. If not, the opportunity to perform the diagnostic procedure is generally lost. As a case in point: failure to set aside fresh tissue precludes reliable fluorescence-activated cell sorter (FACS) analysis routine and necessary for the diagnosis of leukemia and lymphoma. Ideally, then, cells or tissue placed in transport or culture medium for cytogenetics is important. Frozen tissue is perhaps even more important, as all manners of molecular diagnostics (particularly for RNA) are now possible. Small fragments in cryopreservative, if available, are invaluable for a “second look” in the future if needed (a nice way to compare pretreatment versus posttreatment tumor for chemosensitivity, perhaps, or especially new methods such as gene expression profiling, which is discussed under Gene Expression Profiling by DNA Microarrays). Also, a fundamental technique such as EM, still a mainstay for childhood tumor diagnosis, requires fixation in glutaraldehyde for optimal interpretation.

If these procedures are followed, virtually every diagnostic modality available now and in the future is likely to be feasible. In reality, experience to date under even the most rigorous circumstances (e.g., protocol admissibility contingent on submission of tissue in this fashion) has rarely succeeded in much more than 50% of cases. This has led to the development of diagnostic techniques that provide similar information but obtain it from routinely fixed and processed tissue. Thus, cytogenetic analysis for a chromosome translocation can now be substituted by polymerase chain reaction (PCR) analysis. [20] Gene amplification can be detected by fluorescent *in situ* hybridization (FISH). [21,22] and [23] Antigen retrieval methods allow monoclonal antibody–mediated detection of scant antigens otherwise detectable only by FACS. [24] Although vital to ensure every case can benefit from advanced diagnostics necessary for optimal therapy, these alternative methods sometimes introduce serious doubt into the results: what if the PCR is negative? What if the FISH analysis is plagued by high background formalin-induced fluorescence, obscuring the signal? What if the immunochemical results are equivocal? Most cases succeed despite these handicaps, but it is clear that adherence to a protocol, as described in

Figure 8-6, can avoid all these pitfalls.

### Interplay of Multiple Diagnostic Techniques in Tumor Diagnosis

The fundamental reason for the multiple diagnostic approaches advocated in Figure 8-6 is that no method alone suffices for all tumors. Childhood cancer diagnosis (and all pathologic diagnoses, for that matter) is better viewed as a contingency tree. *If* the initial results suggest certain alternatives, *then* appropriate ancillary diagnostics are to be used. This is especially true in the current environment of limited resources for patient diagnosis and treatment. Diagnostic procedures cannot be used for research interest alone, and most DNA or RNA diagnostics are not reimbursable from third-party payers, despite their proven utility in patient management. This state of affairs mandates frugal application of only the most relevant diagnostic methods.

The typical childhood cancer case in our institution, as in other children's hospitals, is handled as illustrated in Figure 8-6. *After* the routine formalin-fixed, hematoxylin and eosin (H&E) slides are returned and examined, the diagnosis is either clear (perhaps half the time) or more studies are required. In many cases, the diagnosis is clear, but protocol requirements mandate special studies, such as *MYCN* and ploidy studies in NB. This tissue is submitted either at the outset if the diagnosis is unequivocal or after the initial studies document the expected (or unexpected!) diagnosis. In the latter case, failure to follow the guidelines in Figure 8-6 may seriously compromise diagnosis. If diagnostic uncertainty remains, a host of methods remains that in virtually every case will result in a specific and reliable diagnosis, generally with an estimate of clinical aggressiveness. These common methods are summarized in Table 8-1. Note that these methods are used judiciously, such that only the rare diagnostic dilemma will invoke most or all of these methods. Nonetheless, the availability of these methods, especially the newer molecular genetic methods (note that five of ten are molecular), has transformed the field of childhood cancer diagnosis from one littered with misdiagnoses and misleading information to an orderly, precise, and generally correct one, tightly linked to therapeutic protocols that depend on this information for patient protocol eligibility and treatment. This is rather unique to childhood cancer and is the fundamental reason for a detailed consideration of the optimal diagnostic evaluation of the child with cancer.

| Method | Comment |
|---|---|
| Light microscopy | Mandatory for all cases |
| Immunohistochemistry | First choice ancillary diagnostic; widely used |
| Molecular generic: reverse transcriptase polymerase chain reaction | Most common molecular diagnosis; now routine |
| Molecular generic: FISH | Rapidly supplanting cytogenetics for many cases |
| Molecular generic: *in situ* hybridization | Specialized use to date (nontumor; Epstein-Barr virus typical) |
| Special stains | Still needed in some cases; useful and cheap |
| Electron microscopy | Still widely used to augment light microscopy |
| Cytogenetics | Needed when no suitable FISH probes available |
| Molecular generic: SKY, CGH | SKY soon to be a useful diagnostic; CGH maybe |
| Molecular generic: DNA sequencing | Rare case such as Li-Fraumeni syndrome (p53 mutation) and others |

CGH, comparative genomic hybridization; FISH, fluorescent *in situ* hybridization; SKY, spectral karyotyping.

**TABLE 8-1. TOP TEN DIAGNOSTIC METHODS FOR TUMOR DIAGNOSIS**

## CHILDHOOD TUMOR DIAGNOSIS

### Character of Childhood Cancer

As noted earlier, childhood cancer presents certain diagnostic challenges. In addition to the unusual variety of age-dependent tumors compared to adult cancer, the typical childhood tumor either lacks definitive morphologic evidence of lineage, or histogenesis, (e.g., small blue cell tumors) or the lineage is ambiguous (e.g., spindle cell tumors). This ambiguity in diagnosis, coupled with the need for definitive diagnosis to establish a treatment regimen, necessitates the use of a variety of diagnostic methods far beyond H&E slides. For example, a specific diagnosis, such as NB, is insufficient; the prognostic subgroup, as determined by the Shimada, now International, classification (Fig. 8-7),[25,26] as well as ancillary studies, such as *NMYC* status by PCR (Fig. 8-8) and FISH (Fig. 8-9), TRKA expression (Fig. 8-10), 1p deletions, and ploidy, are also mandated or requested for entry on current protocols. [27] But what if the simple tumor diagnosis is not possible by H&E study alone? This problem, rare among adult cancers, is frequent in childhood cancer cases and warrants some consideration.

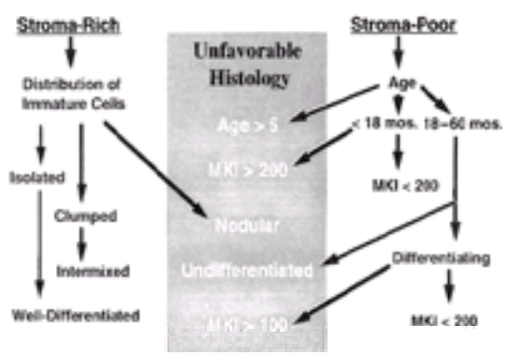


**FIGURE 8-7.** Shimada classification system. Although various versions of the standard classification of childhood neuroblastoma based on morphology and associated prognosis have been published by several authors, this version simplifies the classification by describing two basic groups, stroma-rich ( *left*) and stroma-poor (*right*). Only one stroma-rich type is associated with a poor prognosis ( *gray box*), with clusters or nodules of immature cells set against a well-developed, poorly cellular stoma. In contrast, only two forms of stroma-poor tumors have a favorable prognosis, those in patients younger than 18 months with a mitotic-karyorrhectic nuclei index (MKI) of less than 200, and those in patients aged 18 to 60 months with differentiating tumor cells and an MKI less than 200. All other types in this group are associated with a poor prognosis, as indicated. (Adapted from Shimada H, et al. Histopathologic prognostic factors in neuroblastic tumors: definition of subtypes of ganglioneuroblastoma and an age-linked classification of neuroblastomas. J Natl Cancer Inst 1984;73:405–416.)

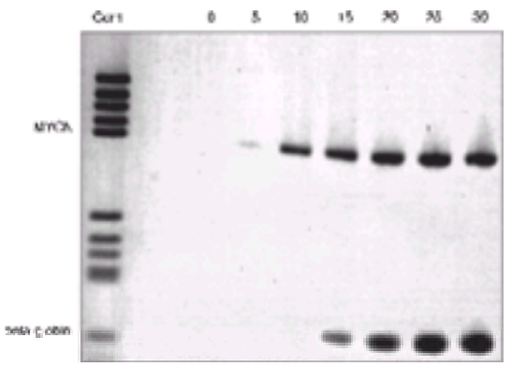


**FIGURE 8-8.** Polymerase chain reaction (PCR) analysis to detect *MYCN*. DNA from a minute patient sample was simultaneously amplified by PCR with primers for both *MYCN* and a control gene, beta globin, as indicated. Product was visible after even five cycles for *MYCN*, whereas beta globin was detectable only after 15 cycles, indicative of *MYCN* gene amplification. (Courtesy of J. Peters, Pathology Department, Children's Hospital, Los Angeles.)

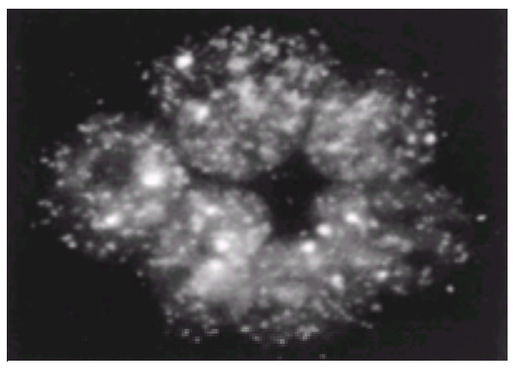

**FIGURE 8-9.** Fluorescent *in situ* hybridization analysis of *MYCN*. In this imprint of a neuroblastoma, a rosette of six cells is detectable. Each nucleus contains two or more bright signals, corresponding to copies of chromosome 2, the normal site of *MYCN*. Conversely, the multitude of fainter signals represent *MYCN* copies, numbering in the hundreds per cell. Even after the normal chromosome 2–based copies of *MYCN* are subtracted from the total, there is a gross amplification of *MYCN* in this tumor. Using this method, results are available within 1 or 2 days of biopsy, far faster than with Southern blot methodology. Fluorescein, ×630.

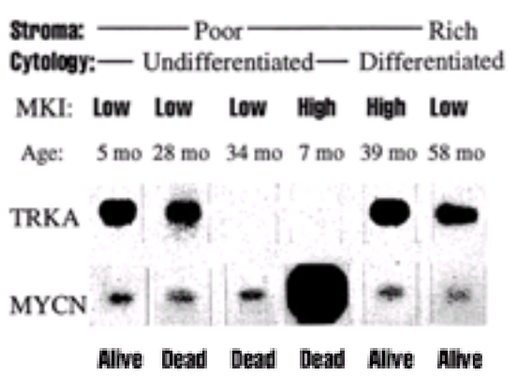


**FIGURE 8-10.** Polymerase chain reaction analysis of *TRKA* and *MYCN*. In this composite blot of *TRKA* (high-affinity nerve growth factor receptor) expression (*upper row*) and *MYCN* copy number determined by Southern blot (*bottom row*), the outcome is compared with Shimada classification. Patients with single-copy *MYCN* that express *TRKA* generally survive. The exception in lane 2 was associated with a poor-prognosis Shimada category. Sequence analysis of this *TRKA* product revealed an 18-bp deletion that rendered the gene product (a membrane tyrosine kinase receptor) biologically unresponsive to nerve growth factor, the functional equivalent to no *TRKA* expression. Conversely, patients with single-copy *MYCN* who lack *TRKA* expression (*lane 3*) have a poor prognosis, as do those with amplified *MYCN* (*lane 4*). Prognosis therefore correlates more closely with *TRKA* expression of the neuronal isoform than with *MYCN* stats and is best with the Shimada classification system. MKI, mitotic-karyorrhectic index. (Courtesy of H. Shimada, Pathology Department, Children's Hospital, Los Angeles.)

## Small Round Cell Tumors of Childhood

The name for this group of difficult-to-diagnose childhood tumors, small round cell tumors (SRCTs) of childhood, refers to the usual appearance of these lesions ( Fig. 8-11). A typical list is found in Table 8-2. Various authors might include other entities, but the point remains the same: they are often largely indistinguishable from each other histologically. As a group, they are primitive or embryonal in appearance, often present in misleading clinical situations (i.e., bone marrow metastases from an occult primary), and lack any particular morphologic features that would allow a precise diagnosis. As a result, they have engendered an entire literature on this subject.[7,8,28]

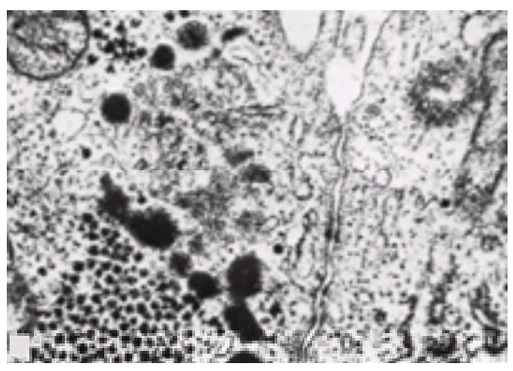

**FIGURE 8-11.** Morphology of typical small round cell tumor. Routine histologic or cytologic examination is of little value in perfectly undifferentiated tumors as illustrated here. Despite high-quality histology and staining, there are no discernible morphologic clues that would allow an unequivocal diagnosis. This so-called small round cell tumor problem is prevalent among childhood tumors and is one of the reasons for the widespread adoption of ancillary diagnostics to provide unequivocal and precise diagnosis.

| |
|---|
| **Small round cell tumors** |
| Peripheral primitive neuroectodermal tumors (PPTs) |
| Ewing's tumor |
| Peripheral neuroepithelioma |
| Askin's tumor |
| Primitive malignant ectomesenchymoma |
| ? Olfactory neuroblastoma |
| RMS |
| Alveolar RMS |
| Embryonal RMS |
| Undifferentiated sarcoma |
| Neuroblastoma |
| Intraabdominal desmoplastic small round cell tumor |
| Malignant lymphoma |
| Anaplastic large cell lymphoma |
| Burkitt's lymphoma |
| Malignant melanoma of soft parts |
| Small cell osteosarcoma |
| Extra-renal monophasic Wilms' tumor |
| Extra-renal rhabdoid tumor |
| **Spindle cell tumors** |
| Congenital/infantile fibrosarcoma |
| Adult-type fibrosarcoma |
| Fibromatosis |
| Infantile fibromatosis (including aggressive fibromatosis) |
| Other forms of fibromatosis |
| Myofibromatosis (including infantile hemangiopericytoma) |
| Synoviosarcoma |
| Malignant fibrous histiocytoma/primitive sarcoma not otherwise specified |
| Spindle cell RMS |
| Malignant schwannoma and other nerve sheath tumors |

RMS, rhabdomyosarcoma.

**TABLE 8-2. DIFFERENTIAL DIAGNOSIS OF PEDIATRIC BONE AND SOFT TISSUE SOLID TUMORS**

Because these tumors often cannot routinely be morphologically distinguished from one another, other diagnostic methods must be used to establish a reliable diagnosis. They are a model system that demonstrates the benefits of a multimodality approach to tumor diagnosis. These nonmorphologic techniques noted earlier and discussed in following sections in detail provide not only diagnoses but also prognostic information. By far the most interesting and informative methods to have emerged in the past several years are genetic.[7,18,29,30] and [31] Furthermore, the emerging methods, also discussed in the section Diagnostic Methodology are ever more sophisticated genetic methods that for the first time offer the prospect of predicting not only a diagnosis but also parameters such as biologic behavior, propensity for

metastasis, and drug sensitivity, to name only a few.[32,33,34 and 35] This will likely usher in an era of customized therapy based on these data.

One cautionary note is that a common belief, first espoused with the advent of “sophisticated” diagnostic methods, such as EM and immunohistochemistry, was that “conventional” diagnostic methods, such as routine H&E studies, would be eliminated in favor of these methods. To paraphrase Mark Twain, the rumors of the death of H&E pathology are greatly exaggerated. No other method returns so much information for so little expenditure of time and money. The real issue is that H&E analysis is no longer sufficient in the majority of cases. Thus, the following discussion *presumes* that the diagnostic workup begins with routine H&E studies, followed as indicated by the methods discussed. Any other sequence is generally unacceptable and leaves the diagnostician liable for medicolegal sanction. Cancer diagnosis requires an initial histopathologic tissue examination.

## Diagnostic Methodology

The basic approach to tumor diagnosis that we employ is as follows, using the methods listed in Table 8-1. A representative tissue block is submitted for formalin fixation, paraffin embedding, and routine H&E stain. If, on microscopic examination, there is any diagnostic ambiguity, a series of special studies are chosen based on the differential diagnosis. The most common secondary diagnostic procedure is immunohistochemistry. Concurrent with, or subsequently, special stains [e.g., periodic acid–Schiff (PAS)], EM, or molecular studies are used, depending on the situation. Finally, the entire set of findings are reviewed and interpreted in context with the clinical history and H&E diagnosis to arrive at a final diagnosis. In virtually every case, no matter how undifferentiated, the typical childhood tumor yields to this diagnostic armamentarium. Very few diagnoses are reversed when such an extensive diagnostic evaluation is undertaken. Conversely, H&E diagnoses are often overturned. Thus, it is prudent to use the many ancillary diagnostic methods available to the pathologist with judicious discretion.

Although most tumors require only a few of the procedures listed, some cases are candidates for all of them. This decision to proceed is generally based on the results of the routine H&E studies, although many tumor groups require specific studies that are initiated at the time of initial receipt of the specimen, regardless of diagnostic status. If this is not done, nothing is lost, as long as the procedures diagrammed in Figure 8-6 are followed at the outset.

Before proceeding to a detailed discussion of useful diagnostic methods, it is worthwhile to briefly review the basic methods required for optimal light microscopic diagnosis. With increasing emphasis on other diagnostics, an inevitable loss of attention to detail in the most fundamental workup can occur. We have seen entire specimens procured for studies, such as FACS analysis, only to discover in the aftermath that the tumor in question was never properly diagnosed, is not a leukemia or lymphoma, and never even had basic H&E pathology. This should be avoided. Some attention to the basic methods is in order.

### *Light Microscopy*

No specimen is more widely studied and available for additional analyses than the routine formalin-fixed, paraffin-embedded tissue sample. Accordingly, stringent attention should be paid to a few simple precautions. When performed properly and with attention to quality of the final product, light microscopy provides sufficient evidence for diagnosis in the majority of cases; the better the quality, the less the need for the techniques discussed in the following sections. This is illustrated in Figure 8-12, in which sections from the same case are illustrated. Initial sections were of poor quality and were nondiagnostic ( Fig. 8-12A). When additional sections were carefully cut and stained, the identity of tissue elements and therefore the diagnosis became apparent ( Fig. 8-12B). Furthermore, many new diagnostics have and are being developed to use the ubiquitous specimens. They will fail if simple procedures such as handling, fixation, embedding, and storage are haphazardly applied.

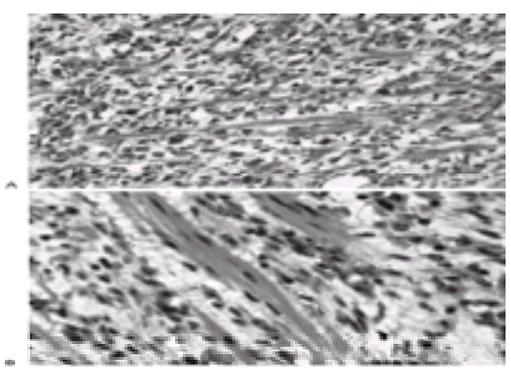

**FIGURE 8-12.** Poor and well-done histology of a typical tumor. **A:** The appearance of a soft tissue neoplasm in a 9-month-old infant. The identity of bands seen coursing across the photograph was difficult to distinguish as fibrous tissue, myofibroblastic elements, or smooth muscle. The correct interpretation only became apparent with proper fixation and embedding, as seen in **(B)**, in which the correct identity as well-differentiated smooth muscle is evident. The surrounding primitive mesenchymal tumor (embryonal rhabdomyosarcoma) is now also evident. Most diagnostic problems in childhood tumor interpretation benefit from optimal-quality histology. Morphology remains the most useful and necessary first step in diagnosis and the subsequent choice of other ancillary diagnostics.

At the outset, tissue should be handled at least in a clean environment with clean instruments, but preferably in a sterile environment such a laminar flow hood if available. *After* tissue is chosen for special studies (Fig. 8-6), including touch preparations and blocks of tissue frozen in optimal cryopreservation temperature compound, if sufficient tissue is available, routine tissue specimens should be submitted in cassettes no thicker than 3 mm. If rapid processing is required, thinner specimens are necessary. Representative sections of large specimens are important, as these tumors are frequently heterogeneous. Some pathologic studies, such as the International (Shimada) classification of NB, cannot be reliably performed on limited specimens such as needle biopsies. When tissue is available, a block for every 30 to 50 g of tissue is a prudent amount to include, although these decisions should be driven by the gross examination. Fewer blocks will suffice in large, homogeneous tumors such as Wilms' tumor. Tumors with obvious heterogeneity on cut surface should be multiply sampled to include all apparent areas, regardless of size.

Optimal fixation is mandatory for many studies, from routine H&E to immunohistochemistry and molecular genetic methods. We recommend a high-quality, buffered 4% formalin fixative for all routine light microscopic studies. If other fixatives are used, they should only be used on additional blocks of tumor tissue. Failure to fix at least one tissue fragment in formalin is a regrettable mistake, to be avoided at all costs. Given the increasing use of formalin-fixed tissue for molecular diagnostics combined with the proven utility of these tissues for diagnosis, there is no other single procedure that better guarantees successful diagnosis.

Once fixed adequately (i.e., at least several hours for any tissue fragment between 1 and 3 mm in thickness), tissues should be embedded in good quality, reasonable (i.e., low) melting point paraffin or paraffin/plastic hybrid to preserve antigenicity of the tissue. Sections should be cut onto acid-cleaned glass slides, if possible, to ensure good adherence, especially because many routine slides are not used for decidedly nonroutine purposes such as *in situ* hybridization, laser capture microscopy, and *in situ* PCR. Tissue that falls off the slides curtails successful use of these methods. Thin sections, no more than 5 µ, are important for diagnostic precision in a variety of methods. Finally, attention to precise, high-quality staining ( *not* batch-processed slides of marginal quality) is mandatory, although frequently overlooked. The wise pediatric pathologist will not accept such slide material.

Although this procedure may seem self-evident, these simple principles are too often eschewed; review of any group of cases assembled from material submitted from a variety of laboratories makes this painfully obvious. Because of this single oversight alone, many of the techniques to follow are often pursued for lack of high-quality light microscopic information. Often, however, these will have no chance of success due to poor-quality tissue; such tissues often yield little useful information.

### *Immunocytochemistry*

Currently, the most universally used ancillary diagnostic method is light microscopic immunocytochemistry. It is routinely used in the majority of childhood cancer cases to confirm presumed histogenesis (based on presumed lineage-specific markers, although this practice is fraught with exceptions—for example, muscle-“specific” desmin in vessels and myofibroblasts). Nonetheless, when used in tandem with routine H&E, this method has become the preferred adjunct to

establish a rapid (i.e., overnight), precise, and reliable diagnosis in a majority of cases.

A detailed discussion of the immunocytochemical results expected from every antibody and every tumor is obviously beyond the scope of this chapter, and is more appropriately found elsewhere. However, Table 8-3 summarizes some of the more useful and specific antibodies used in most pediatric pathology services, along with their common tumor association.

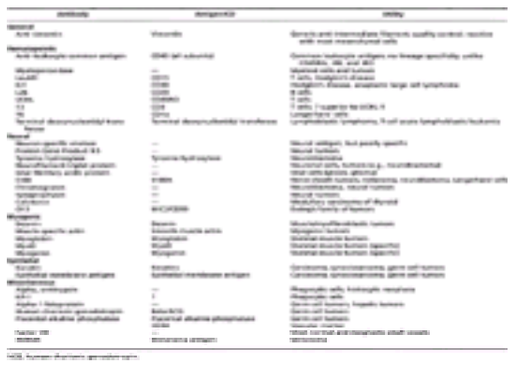

**TABLE 8-3. USEFUL ANTIBODIES IN CHILDHOOD TUMOR DIAGNOSIS**

The most important point concerning the results listed in Table 8-3 is that they are only a guide to expected results; they are not individually always reliable, and anomalous results are the rule, not the exception. The more antibodies used in a given case, the greater the probability of an untoward result. Thus, we interpret the results of immunocytochemistry only in context with light microscopy. Inappropriate results are viewed with skepticism.

A few specific comments about the behavior of some of these antibodies in specific settings are in order. The following discussion of specific genes is based on actual experience of a group of pediatric pathologists in a children's hospital. All diagnostic immunohistochemistry ordered over the course of the year 2000 was compiled and all antibodies used other than sporadically for tumor diagnosis were tallied as Table 8-3. Other pathology groups will likely use some other antibodies with different frequency, but overall this is a reasonable representation of useful antibodies for tumor diagnosis. Thirty-five antibodies are sufficient in our hands for all common tumors. Others are used in a research setting, and promising new antibodies are constantly evaluated. Nonetheless, this tabulation reflects the current state of diagnostic immunohistochemistry.

A second general point is that the overall pattern of antigen localization in a tissue depends on several factors, most notably the cellular disposition of the antigen. Three basic patterns are observed: nuclear, cytoplasmic, and cell surface. These are illustrated in Figure 8-13A, Figure 8-13B and Figure 8-13C. If a tissue shows positive staining but of an inappropriate pattern, the result is highly suspect and should be used with caution. Similarly, artifacts such as geographic staining patterns (e.g., areas of positivity and negativity across a section, independent of tumor heterogeneity), staining of acellular areas (e.g., stroma), and staining of normal cellular elements that should not stain (e.g., endothelial cells for neural markers) are routinely encountered and must be factored into the interpretation. Rarely is a section free of all artifacts. The results, then, depend on the experience and skill of the diagnostician.

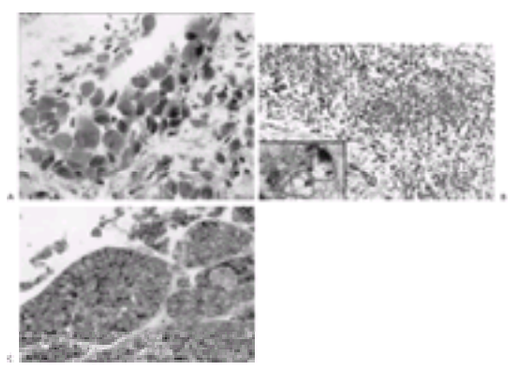

**FIGURE 8-13.** Immunohistochemistry—typical results. Immunohistochemistry is widely used in surgical pathology of adult and childhood tissues. Many classes of antigens (e.g., nuclear, cytoplasmic, and cell surface) are widely detected in the evaluation of childhood cancer. Illustrated here are three categorical examples. **A:** An example of nuclear staining for a transcription factor, myoD, used to detect extremely undifferentiated childhood rhabdomyosarcoma. **B:** Vimentin, a ubiquitous intermediate filament found in most childhood tumors, localized to the cytoplasm (*detail, see inset, lower left*). **C:** *MIC2*, a cell surface antigen found in Ewing's sarcoma and T-cell lymphoma and leukemia.

### Vimentin

Vimentin, a 10-nm intermediate filament, is expressed in all cells of the body at some developmental stage and at some level.[36,37] Antibodies to vimentin have no specific diagnostic utility. It is, however, the single most useful antibody for quality control, the most common reason for incorrect or invalid results by this methodology. Epithelia are generally negative, but most other cells are positive. Within a given section, *something* will be reactive (often blood vessels and fibroblasts, for example), thereby proving that the antigenicity of the section is intact and enabling reasonably reliable interpretation of results with other antibodies.

### Leukocyte Common Antigen, or CD45, Nonrestricted

CD45 is an abundant cell surface glycoprotein on immune cells that functions as a phosphotyrosine phosphatase.[38] All hematopoietic cells should in theory react with this antibody. That is not the case with certain lymphomas,[39] for example, possibly because loss of CD45 may be associated with the development of aggressive T-cell leukemias,[40] but most lymphomas, leukemias, and granulocytic sarcomas do react. Hodgkin's disease is poorly reactive.[41] Many commercial sources exist, generally with similar if not identical reactivity, despite the fact that most are single monoclonal antibodies (or, less often, “cocktails” of several anti-CD45 monoclonal antibodies). Because benign *and* malignant hematopoietic cells react with the antibody, it is most useful as a tool to distinguish one hematopoietic malignancy from another, nonhematopoietic tumor. It is of no value (in general) in distinguishing benign, reactive, or malignant hematopoietic cells from one another.

### Myeloperoxidase

Although myeloperoxidase activity can be detected histochemically in suitable specimens by the generation of free oxygen from peroxide, the enzyme itself, a protein, can be detected with suitable antibodies.[42,43] This detection is especially useful when discrimination between a granulocytic neoplasm (typically either acute myelogenous leukemia or granulocytic sarcoma or chloroma). Both benign and malignant myelocytic lineage cells are reactive, although highly immature myeloid precursor cells may be weakly reactive.

### LeuM1 (CD15)

Despite the name, LeuM1, reactive with the CD15 cell surface antigen, identifies predominantly T cells and Hodgkin's disease Reed-Sternberg cells.[44,45] Although useful, it does not distinguish the two. It is best used in conjunction with CD30 antibodies, with which it can reliably distinguish Hodgkin's disease (positive) from

anaplastic large cell lymphoma (ALCL; negative).

**Ki1 (CD30)**

Ki1, part of the Kiel University series of monoclonal antibodies (thus, the name, Ki1), has been most widely used to identify Hodgkin's cells in Hodgkin's disease. [46,47] and [48] However, it also reacts with ALCL in children. [49] Concurrent use of anti-CD15 antibodies (see the previous section) distinguish the two: Hodgkin's cells are reactive with both; ALCL reacts only with CD30. Because of overlapping immunophenotypic features of the two, most authors employ molecular diagnostics (see the section on molecular genetic diagnostic techniques) to detect the NPM/ALK chimeric gene found in ALCL. [50] Despite this, there are reports of Hodgkin's disease with the fusion gene, [51] or that evolve into ALCL, so this distinction may not always be possible.

**L26 (CD20)**

Recognized as a pan-B cell–reactive monoclonal antibody useful in paraffin-embedded specimens from the outset, [39,52] it was soon determined that L26 recognized a known pan-B antigen, termed *CD20*. [53] This antibody has endured as the single most useful marker of B cells in general, and neoplastic ones in particular, despite occasional staining of T cells. [54,55] and [56] In conjunction with CD45, it will stain B cells even when CD45 (LCA) is negative.

**UCHL-1 (CD45RO)**

The counterpart to the pan-B antibody L26 is UCHL-1, also recognized early on as highly specific for T-cell lineage and reactive with paraffin-embedded tissues. [57,58] and [59] It is therefore the ideal companion to L26 for T- versus B-lineage determination in hematopoietic neoplasia. The only drawback is a more frequent occurrence of negativity (i.e., 61% to 78%) in bona fide T-cell malignancies. [57,60] Despite these inconsistencies (true of all antibodies and their target antigens to a greater or lesser degree), the panel of CD45, CD20, and CD45RO is the standard set in routine use for hematopoietic and T- versus B-lineage studies.

**T3 (CD3)**

Described earlier than the other major T-cell marker CD45RO, [61,62] anti-T3 antibodies (e.g., UCHT-1, OKT3) have been used in both flow cytometry to mark T cells and subsequently in solid hematopoietic tumors as well, especially because the T3 epitope, CD3 (actually a group of transmembrane glycoproteins), [61] survives formalin fixation and paraffin embedding. [63] In practice, UCHL-1 is more often used, but anti-CD3 antibodies are valuable adjuncts, especially because a significant percent (as much as 20%) of T-cell malignancies fail to react with UCHL-1. [58]

**CD1a (T6)**

Antibodies against the CD1a epitope were originally recognized to react with lymphoid cells of T-cell lineage, specifically mid-thymocyte lineage. [64] Common acute lymphoblastic leukemia cells were also noted to be reactive in a high percentage of cases, but not sufficiently consistent to denote T-cell lineage in this malignancy. [65] At approximately the same time, it was noted that anti-T6 (CD1a) antibodies reacted consistently with the Langerhans' cells of histiocytosis X, or Langerhans' cells histiocytosis by current terminology. [66] Further investigation revealed that normal Langerhans' cells in epidermis and lymph node are also reactive (typically, $CD1a^+/HLA\text{-}DR^+$), documenting the Langerhans' cell lineage of normal and neoplastic histiocytes. Not surprisingly, malignancies of follicular dendritic cells and interdigitating reticulum cells (e.g., interdigitating reticulum cell sarcomas) were also found to be reactive, [67] although confirmation optimally requires comprehensive immunophenotyping, as some malignancies of histiocytic lineage lack the usual phenotype of Langerhans' cells. [68] At a practical level, anti-CD1a antibodies in conjunction with anti-S100 antibodies are of great value in confirming a diagnosis of Langerhans' cell histiocytosis, a not uncommon problem in childhood tumor diagnosis. $CD1a^+/S100^+$ cells are unequivocally of Langerhans' cell lineage, although specific features, such as pure lymph node involvement and sarcomatous appearance, may suggest a histiocytic sarcoma or interdigitating cell sarcoma, which are also generally positive. [68]

**Terminal Deoxynucleotidyl Transferase**

Although terminal deoxynucleotidyl transferase (TdT), a DNA polymerase, was first detected on the basis of its enzymatic activity (specifically in adding nontemplated nucleotides during T-cell receptor gene rearrangement), whereby a strong correlation with T-cell lineage was noted, [69] it was quickly realized that TdT activity is therefore a reliable index of T-cell lineage among lymphoid neoplasia. [70,71,72,73] and [74] Many studies based such detection on enzymatic assays, [71,73,74] but less cumbersome methods designed to detect the enzyme itself independently of its enzymatic activity were soon developed. [75,76] These methods were largely immunofluorescent in character, but with the general advances in immunohistochemistry true of the past two decades, these antibodies have been adapted to chromogenic avidin-biotin–based methodology, specifically for use on paraffin-embedded, formalin-fixed tissues. [77,78] and [79] Comparative studies confirmed both a close correlation with the enzymatic activity and the association with acute lymphoblastic leukemia and lymphoblastic lymphoma. [80,81] As is virtually universally the case with antigen expression on cells and tissues in general, even TdT activity has now been identified on non–T-cell neoplasia, [82] and rare cases of TdT-lymphoblastic leukemias have been reported. [83] Despite these caveats, antibody-based TdT detection remains the single most useful means of detecting T-cell leukemia and lymphoma (notably lymphoblastic lymphoma) in children.

**Neuron-Specific Enolase**

Neuron-specific enolase (NSE), the so-called neuron-specific isozyme of a family of endase enzymes, was thought to be restricted to neural tissues and tumors, and numerous studies at first appeared to support its utility as a diagnostic marker for tumors of neural lineage. [84] This apparent specificity was soon recognized to be incorrect; numerous non-neural tumors were subsequently shown to express NSE. [85] Despite these problems with nonspecificity of detection of the g subunit of NSE, it has subsequently been demonstrated that NSE is nonetheless the single most sensitive and universal marker in neural tumors, despite its proclivity to be expressed in non-neural tumors as well. [86] Although widely used to detect neural phenotype in tissues and tumors, it is generally used in conjunction with antibodies against other neural antigens such as PGP 9.5 and soluble brain-derived protein (S100). [86]

**Protein Gene Product 9.5**

Problems with nonspecificity of NSE prompted a search for other, more reliable markers of neuronal differentiation. Protein gene product 9.5 (PGP 9.5), a soluble glycoprotein isolated from brain, has been widely adopted as an alternative, or in many cases, an adjunct diagnostic antibody in the evaluation of neural tumors. [87] Its diagnostic value in NB was promptly reported [88,89] and compared to NSE staining. It has been found to be more reliably associated with neural tissues but with similar sensitivity. [86] Currently, PGP 9.5 in conjunction with tyrosine hydroxylase (TH) is widely used in the specific diagnosis of NB (other, non-sympathetic neural tissues and tumors are TH negative). [90] Thus, for general purposes, *both* NSE and PGP 9.5 are ideally used in tandem in the diagnosis of any neural neoplasm, notably NB and the EFTs. TH positive will identify the former in contradistinction from the latter.

**Tyrosine Hydroxylase**

Among the many neurotransmitter enzymes necessary for the adrenergic (catecholamine-producing) metabolic pathway, TH has proved uniquely useful as an indicator of NB. [91,92,93] and [94] Elevation of this enzyme, produced only by monoaminergic neurons of the sympathetic ganglia (the site of virtually all childhood NBs), is closely associated with serum and urinary excretion of intermediary metabolites homovanillic acid and vanillylmandelic acid. [95,96] However, not all NBs show increased expression of this enzyme. [97] Consequently, detection of TH activity may be diagnostic, but lack of activity does not preclude a diagnosis. The presence of TH has been assessed immunochemically, and a strong association between detection of the proteinaceous enzyme and TH activity has been noted. [98,99,100] and [101] Reverse transcriptase-PCR (RT-PCR) assays have also been developed, [102,103,104] and [105] but immunochemical detection appears to be at least as useful, and offers the additional advantage of tissue localization. [106] The specificity of TH expression is well documented, and thus detection of TH by immunohistochemistry is highly specific and generally positive in NB. [90,107]

### Neurofilament Triplet Protein

Neurofilament triplet protein, so called because three different transcripts and resulting different molecular weight subunits are expressed to produce the final intermediate filament of neural tissue, is routinely expressed in the classic neural tumors of childhood, NB and medulloblastoma. [108,109,110 and 111] It is also occasionally detected in the peripheral neural tumors, now collectively referred to as the EFTs, but less reliably so. [112,113] Furthermore, all intermediate filaments can show lineage-inappropriate expression in tumors; NFTP is no exception, typically being expressed in RMS and rhabdoid tumor, to name only two examples. [113,114,115,116 and 117] When used in conjunction with other neural markers like NSE and PGP 9.5, however, NFTP can be a useful marker to document neural lineage in a tumor. It represents definitive evidence of such differentiation, much as myogenic filaments in muscle tumors document myogenesis.

### Glial Fibrillary Acidic Protein

Glial fibrillary acidic protein, normally restricted in its expression to the glial cells of the brain, [118] has nonetheless been documented in a variety of tumors other than gliomas, notably NB, primitive nerve sheath tumors, and even Ewing's tumors. [119,120 and 121] Although most useful when used in concert with NFTP and vimentin, it is occasionally useful separately to document support cell differentiation (e.g., Schwann cells), in conjunction with S100, in NB (so-called stroma-rich tumors), in which its expression is inappropriate but a useful marker of non-neuronal differentiation in this tumor.

### Soluble Brain-Derived Protein

S100 immunoreactivity has long been recognized as a reliable index of neural differentiation. [122] However, it should be noted that S100 is immunoreactive in a broad range of tumors, including neural, nerve sheath, melanocytic, cartilaginous, and Langerhans' cell histiocytic. [123,124,125,126 and 127] Despite this widespread occurrence of immunoreactivity, S100 remains useful in the identification of these cell types. Positivity, then, can only be used diagnostically in context with other features of a given cell lineage in a tumor. For the most part, that is not difficult. S100 immunostaining merely provides confirmation of such lineage in most cases.

### Chromogranin, Synaptophysin, and Calcitonin

Chromogranin, synaptophysin, and calcitonin are selectively expressed in neural tissues (or neuroendocrine cells of the thyroid in the case of calcitonin) and are therefore reliable markers of neuroendocrine phenotype in normal and neoplastic cells. At first, highly selective expression was thought to correlate with neuroendocrine cell lineage alone, but more extensive experience has demonstrated many instances of lack of detectable expression in known neuroendocrine tumors and (especially with synaptophysin) expression in non-neuroendocrine tissues. Thus, although useful, this group of markers is not as widely used as those discussed previously. [128,129,130,131,132,133,134,135,136,137,138,139,140 and 141]

### O13 (p30–32 MIC2, CD99)

The otherwise often ambiguous diagnosis of EFTs was revolutionized by the recognition that an antigen on T cells involved in red cell adhesion, E2, was the product of the pseudoautosomal gene MIC2, which in turn is highly expressed in Ewing's tumors. [142,143,144,145 and 146] Within a short time, detection of the MIC2 p30–32 glycoprotein product by suitable antibodies (often either O13 or Leu 7) became the diagnostic standard for these tumors, at least for that brief period after its initial description and before the recognition of the *EWS-FLI1* fusion gene in this family of tumors. [147,148,149 and 150] Subsequent studies have documented the expression of CD99 in other tumors, most notably some desmoplastic SCRTs (DSRCTs), RMSs, brain tumors, and even angiomatoid fibrous histiocytoma. [141,151,152]

### Desmin

Desmin, a prototypical intermediate filament protein (along with vimentin, NFTP, glial fibrillary acidic protein, and keratin), is strongly associated with myogenic cell differentiation. [118,153] Consequently, it was promptly identified as a potential, easily detectable marker of early myogenesis, and in fact has become the diagnostic standard for detection of skeletal muscle differentiation in suspected RMS, despite its known occurrence in smooth muscle and even myofibroblasts. [154,155,156,157 and 158] More troubling, desmin expression has been reported in completely unrelated tumors [e.g., NB and primitive neuroectodermal tumors (PNETs) as well] thereby undermining its utility as a stand-alone marker of rhabdomyogenesis. [159,160] For these reasons, current practice is to use at least two markers, nominally desmin and muscle-specific actin (MSA). [158]

### Muscle-Specific Actin

Among the several actins (cytoplasmic, skeletal muscle, and cardiac muscle), MSA represents the single most common transcript during normal myogenesis. [161,162] It was therefore a predictable application to develop suitable antibodies against this particular actin and to use such antibodies in the diagnosis of RMS. [163] When compared to desmin, MSA antibody staining has proved a reliable adjunct, but it, too, stains nonrhabdomyogenic cells such as vascular smooth muscle. [164] As long as normal cellular components can be excluded, MSA, preferably in conjunction with desmin, is a potent diagnostic tool for the diagnosis of RMS. [165]

### Myoglobin

Myoglobin, as its name implies, is an oxygen-carrying heme protein normally localized to skeletal muscle and should therefore be a reasonable marker of skeletal muscle differentiation. This fact was recognized early in the evolution of diagnostic immunohistochemistry and used for the diagnosis of RMS. [156,166,167 and 168] Experience over many years has clearly indicated that myoglobin staining is not always reliable, however, and therefore results should never be used in isolation. Ideally, the combination of desmin, MSA, and myoglobin staining optimizes the sensitivity and specificity of immunohistochemical diagnosis of RMS. [169]

### MyoD and Myogenin (Myogenic Transcription Factors)

The exquisite, precisely orchestrated steps that eventuate in the creation of a skeletal muscle cell from an undifferentiated mesenchymal stem cell have been worked out in remarkable detail. [170,171,172,173,174,175,176,177,178 and 179] It is now clear that a few master control genes, notably myoglobin D1 (MyoD1), Myf5, and Myogenin (myf4), all members of the basic helix-loop-helix family of nuclear transcription factors, are responsible for commitment and subsequent differentiation in mesenchymal (or any other) cells. [177,178 and 179] This, then, is the critical first step leading to skeletal muscle differentiation in normal and neoplastic mesenchymal cells. [173,178,180,181] The latter property thus defines RMS at a molecular level. As such, it thus requires detection of these scant nuclear proteins. This has been accomplished, even on routinely fixed tissues, using antigen retrieval methods such that MyoD1 and Myogenin can be readily identified, a finding tantamount to a diagnosis of RMS if the tumor in question is a soft tissue sarcoma of childhood. [24,182,183 and 184] Currently, detection of MyoD or Myogenin in tumor cells is sufficient for a diagnosis of RMS, even in the absence of muscle-specific proteins such as MSA, myosin, or desmin, all of which are expressed later in muscle development.

It is tempting to ask whether there is an even more proximal determinant of muscle lineage in normal and tumor cells. There is, but it is of no value in this context, as the genes responsible, PAX3 or PAX7, are also expressed in neural tissues and certain neural tumors (e.g., the EFTs). Thus, PAX3 or PAX7 expression is not sufficiently specific, even though it is mandatory to incite myogenesis via MyoD and MYF5. [185,186 and 187]

### Keratin

Keratin is the unique intermediate filament of epithelia. [188,189] However, it is also expressed in a number of other tissues and tumors with some frequency. [36,37,190,191 and 192] Consequently, keratin alone does not reliably identify epithelial tumors. It does, however, strongly associate with epithelial phenotypic expression in a tumor in the broadest sense of the word. If one accepts that certain keratins are expressed in all ectodermal and entodermal cells, its expression in even primitive cells of such lineage, such as the EFTs, is not untoward. [193] Likewise, even tumors of clear mesenchymal origin, such as SS, with known ability to undergo glandular differentiation routinely express keratin, even before obvious glandular (epithelial) differentiation. [194,195]

### Epithelial Membrane Antigen

Confirmation of epithelial phenotype in a tumor is thought to be reliably established by concurrent use of anti-epithelial membrane antigen (anti-EMA) antibodies. Coexpression of both keratin and EMA is not observed with any frequency in any cells other than those with epithelial phenotype. In the cases in which both are expressed but no obvious epithelial cells are present, there is increasing evidence for nascent epithelial differentiation, as in monophasic SS and nerve sheath tumors.[194,196,197] Admittedly, exceptions are well known, such as the EFTs, DSRCTs, and leiomyosarcoma, but in general there is strong correlation between epithelial differentiation and coexpression of keratin and EMA.

### Alpha$_1$-antitrypsin and KP1 (CD68) (Phagocyte-Associated Antigens)

Alpha$_1$-antitrypsin (AAT) expression has been associated with phagocytic or histiocytic cells. Experience with antibodies to AAT has been somewhat unsatisfactory, however, as immunoreactivity has been found in a wide variety of cell types.[198,199] This has led to the development of more specific targets found more or less exclusively in phagocytic cells. One in particular, KP1 (anti-CD68), has enjoyed wide use as a reliable marker of phagocytes, histiocytes, and their presumed tumors.[200,201 and 202] Although useful, results are sometimes less than ideal, limiting their utility in the absence of other confirmatory information.[200,201]

### Alpha-1-fetoprotein, Human Chorionic Gonadotropin, and Placental Alkaline Phosphatase (Germ Cell Markers)

Germ cell tumors have for some time been readily diagnosed by detection of specific antigens more or less unique to this group of tumors. The three most commonly used antibodies to detect alpha-1-fetoprotein, human chorionic gonadotropin, and placental alkaline phosphatase have now been well characterized and shown to correlate remarkably well with the germ cell tumor lineage of the tumor in question. Very few exceptions occur. As a result, any tumor suspected of being a germ cell tumor or to contain even elements of germ cell tumor can be documented with a simple panel of three well characterized antibodies.[203,204,205,206,207,208,209 and 210]

### CD34 and Factor VIII (Vascular Markers)

CD34, an antigen nominally present on hematopoietic precursor cells, has also been detected on vascular (endothelial) cells and vascular tumors.[211,212] However, it has also been detected on a variety of other tumor cell types to the point that its utility as a vascular marker has been questioned.[213,214] Recent studies with laser capture microscopy and immunodetection confirm, however, that the antibody detects a genuine tumor cell product of the correct molecular weight.[215] Thus, specificity of antigen recognition is not an issue; rather, the antigen is expressed on diverse types of nonvascular cells as well, thereby undermining the utility of anti-CD34 antibodies as purely vascular markers. Most authors therefore use a combination of anti-CD34 and anti-factor VIII or ulex agglutinin in their efforts to confirm a vascular phenotype to a tumor of uncertain histogenesis. In one recent study of 80 malignant vascular tumors (angiosarcomas), all were at least focally positive for factor VIII–related antigen, whereas only 74% were positive for CD34.[212]

### HMB45 (Melanoma Antigen)

Although melanoma per se is uncommon in children, melanocytic tumors are not so rare, notably melanoma of soft parts (clear cell sarcoma of soft parts) and pigmented peripheral nerve sheath tumor [part of the spectrum of malignant peripheral nerve sheath tumor (MPNST)]. Thus, the introduction of a specific melanoma antigen antibody over a decade ago has been of great (albeit occasional) utility.[216,217,218 and 219] Other antibodies have been proposed, but HMB45 appears to be the most specific and most uniformly reactive among melanocytic lesions.[220,221] The particular value, of course, is in the diagnosis of amelanotic lesions and those with atypical histology (e.g., spindle cell lesions) with very rare false negative results and virtually no false positive results.[222,223,224,225 and 226] The latter point (i.e., specificity) is accentuated by the finding, for example, of composite melanocytic/neuroblastic tumors, wherein only the melanocytic component expresses the HMB45 antigen.[227] Thus, despite limited overall utility, the specificity of this antibody renders it highly useful when the differential diagnosis includes melanocytic lesions of any type.

It is important to remember that the preceding list represents actual everyday usage in a children's hospital. Adult institutions will likely use a very different spectrum of antibodies, reflective of the differences between adult and childhood cancers. The preceding list is of some use in a pediatric cancer setting, however. It is not all inclusive or exhaustive but will account for the bulk of childhood tumors seen in such an institution.

## *Special Stains*

The success of immunohistochemistry and EM has largely replaced the use of special stains in diagnostic surgical pathology. A few remain useful, however, and warrant at least a brief comment here.

The basic special stains in common use, especially the connective tissue stains (i.e., trichrome, pentachrome, and reticulin), are used most commonly to detect a fibrous supporting stroma. This can be useful in determining whether a given tumor is a stroma-producing tumor (e.g., a sarcoma). It can also be useful in distinguishing certain types of sarcoma: At one extreme, hemangiopericytoma generally produces an obvious stroma when stained with a silver reticulin stain, whereas the EFTs do not, providing a simple method of distinguishing the two, which occasionally resemble one another to a striking degree. It should be apparent after reading the molecular diagnostic section that molecular diagnosis has become the gold standard for diagnosis. In the absence of suitable tissue for molecular studies (such as a few submitted unstained slides), a connective tissue stain can be helpful in supporting a diagnostic impression of a stroma-poor sarcoma such as an EFT, as opposed to an hemangiopericytoma, or even a small cell osteosarcoma.

The older diagnostic literature repeatedly refers to the value of PAS, with and without diastase digestion (to remove glycogen), in the diagnosis of childhood tumors.[228,229,230,231 and 232] However, it has also become obvious that tumors that should accumulate glycogen detectable by the PAS reaction sometimes do not, and others that should not, do.[229,231] Thus, the utility of this stain has decreased, but it is still a useful adjunct in confirming the diagnosis of an EFT, for example, while ruling out most lymphomas and NBs.

Finally, myeloperoxidase activity demonstrated histochemically on tissue sections (e.g., von Leder stain) can be an invaluable diagnostic adjunct in the rare case of suspected granulocytic sarcoma, or chloroma.[233,234] Often, these cases present in unusual clinical settings, such as a periorbital mass, preceding any overt peripheral blood abnormalities suggestive of myeloid leukemia.[235,236,237,238,239,240,241,242 and 243] The distinction from more conventional solid tumors of soft tissue, such as Langerhans' cell histiocytosis, lymphoma, RMS, and NB (especially in the periorbital region), is generally easily made, as the von Leder stain is highly specific for myeloperoxidase activity. Although antibodies against myeloperoxidase have been developed,[233] the von Leder stain remains the procedure of choice.

## *Electron Microscopy*

EM no longer plays a significant role in the diagnosis of most adult tumors but is still widely used in the diagnosis of childhood tumors (and other tissues). Thus, although infrequently used in adult settings, approximately 5% to 10% of childhood tumors are studied by EM to improve diagnostic accuracy. The most common reason, beyond diagnostic uncertainty based on ambiguous morphology (the small blue cell tumor problem almost unique to childhood tumor diagnosis), is to clarify conflicting results from other studies such as immunohistochemistry. It is a valuable adjunct, but plays a subordinate role in most diagnostic settings, in which cheaper, more specific, or more rapid diagnostics are used first. Small blue cell tumors are a common setting, but most of these can now be quickly diagnosed by other methods.

Despite these caveats, EM can be indispensable in selected cases. Its greatest contribution is usually definitive evidence of histogenesis, or a tumor-specific ultrastructural feature in which no definitive morphologic or immunochemical feature is identified. Examples include basal lamina in nerve sheath tumor versus fibrosarcoma (Fig. 8-14A), cell adhesions to rule out suspected lymphoma (Fig. 8-14B), Langerhans' granules in histiocytosis X (Fig. 14C), primitive duct or gland formation (e.g., apical cell junctions and basal lamina) in monophasic SS (Fig. 8-14D) or Wilms' tumor, dense core granules and/or neurites in suspected NB (Fig. 8-14E), melanosomes in melanoma of soft parts (Fig. 8-14F) or pigmented peripheral nerve sheath tumors, and Weibel-Palade bodies (usually with numerous endocytic vesicles) in vascular tumors. The actual spectrum of findings in the common (and uncommon) tumors of childhood is of course the subject of an entire literature, too lengthy to review here.[244,245,246,247,248 and 249] Increasingly, the challenge is the availability of this technology, which is both expensive and requires

specialized training for reliable interpretation. These issues increasingly limit the utility of EM for tumor diagnosis.

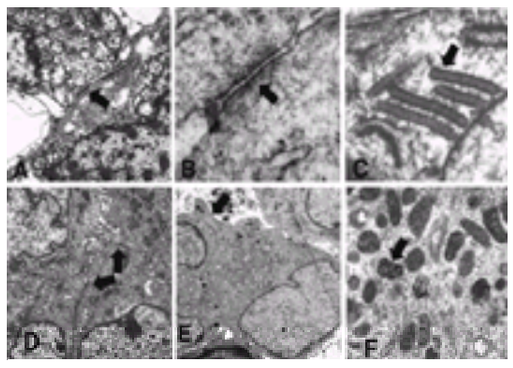


**FIGURE 8-14.** Typical ultrastructure. Electron microscopy still plays a useful role in childhood tumor diagnosis. Six typical findings useful in the diagnosis of childhood cancer are illustrated here. **A:** Basal lamina (*arrow*). Typically found in benign nerve sheath tumors, benign and some malignant muscle, and underlying the glandular elements of synoviosarcoma, basal lamina is a distinctive feature that can help in a differential diagnosis of at least these tumors. **B:** Cell adhesions (*arrow*). When lymphoma or leukemia are a consideration, the finding of any form of cell adhesion, such as that illustrated here, rules out any form of hematopoietic malignancy. This finding is especially useful given the frequent negativity of childhood lymphomas and leukemias for specific lymphoid and T- and B-cell markers. **C:** Langerhans' granules (*arrow*). These structures, of unknown function and origin, are among tumors exclusively associated with Langerhans' cell histiocytosis. They are normally found in dendritic cells in lymph node and in Langerhans' cell of skin. **D:** Gland formation. Although carcinoma is effectively not described in childhood cancer, a typical glandular epithelial formation with apical junctions ( *arrows*) is routinely found in biphasic synoviosarcoma in which islands of nascent glandular differentiation may be identified. **E:** Dense core granules and neurites (*arrow*). A common diagnostic problem in childhood tumors is neuroblastoma versus other small round cell tumors of childhood. The most common differential finding by electron microscopy is the presence of dense core granules and neurites ( *arrow*), which are virtually synonymous with neuroblastoma (although rarely they may be observed in peripheral primitive neuroectodermal tumors and Ewing's tumors). **F:** Melanosomes. Although melanoma is exceedingly rare in children, melanosomes (*arrow*) are routinely found in melanoma of soft parts. This finding can be useful in the differential diagnosis of spindle cell soft tissue tumors in which distinction between melanoma of soft parts, monophasic synoviosarcoma, malignant peripheral nerve sheath tumor, and fibrosarcoma may be problematic.

### *Molecular Genetic Diagnostic Techniques*

For lack of a better term, *molecular genetic diagnostic techniques* as used here applies to the five major methods currently in use to examine the genomic status of a cell or cells: (a) PCR, (b) cytogenetics, (c) FISH, (d) spectral karyotyping (SKY), and (e) comparative genomic hybridization (CGH). The methods fundamentally differ; PCR is applicable largely to extracted DNA or RNA converted into DNA, whereas the other four methods, directly or indirectly, detect whole chromosomal genetic status.

It is also important to note that there are innumerable variations of these methods, and even entirely different methods (e.g., arrays, discussed under Microarrays, Polymorphisms, and Whole Genome Studies). Here we focus on only those methods now in daily use to analyze childhood tumors. Furthermore, specific examples of the application of these general methods to childhood tumor diagnosis are discussed later. The purpose here is to introduce the basic methodology.

#### Polymerase Chain Reaction

PCR is now commonplace, and this discussion is not intended to review the history of this fundamental tool in genetic studies. Modern genomic analysis could not be undertaken without it, nor could most forms of tumor genetics.[250] Rather, the purpose here is to discuss how this basic method has been adapted and used to detect genomic alterations and gene expression in even single cells and paraffin-embedded material, tissue sources never envisioned by those who first used this method. As of this writing, there are at least four basic approaches to the use of this technique: (a) conventional PCR, including detection of mRNA, using reverse transcriptase to create complementary DNA (cDNA) from mRNA (RT-PCR); (b) PCR from paraffin (e.g., short primers); (c) quantitative PCR (as marketed by Roche Pharmaceuticals), and (d) *in situ* PCR on cells or tissue sections. The creative skeptic can certainly identify other methods, including gross amplification of total mRNA extracts from even single cells (as popularized by Eberwine),[250a,250b] but these are not yet in widespread diagnostic use.

Although mRNA can be readily detected and used as an index of gene expression in the analysis of childhood tumors,[251] most diagnostic PCR in childhood tumor diagnosis is used to detect genomic alterations (e.g., gene translocations, amplifications, or deletions). Although this can be performed on genomic DNA, and frequently is, the method of choice in practice is to amplify expressed mRNA sequences. This method is useful, especially when translocation variants generate huge intron sequences that may not be reliably amplified (the FKHR gene, discussed in the section *PAX-FKHR* Gene Fusions in Alveolar Rhabdomyosarcoma, is one example). This works reliably, as documented by an extensive literature on the subject (see references [252,253] and [254] for reviews). However, less than one-half of most tumors are available in a suitable physical state (e.g., fresh, frozen, or imprints) to reliably perform conventional RT-PCR. Thus, the method has been adapted to even paraffin-embedded, formalin-fixed tissues, largely by using primers designed to compensate for the widespread RNA degradation and fragmentation found in these specimens.[255,256,257] and [258]

The other major innovation in PCR diagnostics is the introduction of reliable and seemingly quantitative PCR analysis. Although quantitative PCR analysis is not new, no consensus had emerged as to a preferred method until Roche introduced a commercial platform (Taqman) that routinizes and automates the procedure. This important new tool extends the utility of PCR for detection of gene expression beyond simple presence or absence; quantitative levels can now be measured and compared, as is commonplace for NB and *MYCN* expression (as in index of amplification; see below).

Although premature to stress at this point, it is nonetheless relevant to note the emergence of amplification methods using PCR that offer the possibility of generating abundant RNA that more or less stoichiometrically parallels the abundance of these same species in the native specimen. This method, pioneered by Eberwine,[250a,250b] has been greatly improved in the recent past,[258a,258b] and may well make possible analysis of the entire repertoire of expressed genes by methods such as microarrays and serial analysis of genetic expression (serial analysis of genetic expression; see the section Microarrays, Polymorphisms, and Whole Genome Studies) from the smallest samples, perhaps even single cells.[259] It is not too far-fetched to imagine minimally invasive procedures such as radiographically directed fine needle aspiration or laser capture microscopy of single cells or small groups of target cells (e.g., pure tumor cell populations) coupled with DNA or RNA amplification as a common diagnostic sequence in the future.

#### Karyotypic Analysis (Cytogenetics)

Conventional tumor cytogenetics is in a state of rapid evolution at present. Conventional cytogeneticists are becoming molecular cytogeneticists. Nonetheless, the most common genetic analysis at a whole cell level is ordinary G-banding metaphase cytogenetics, or karyotypic analysis.[260,261] Every case of leukemia and most solid tumors avail of the technology. Most of the diagnostic translocations discussed elsewhere [such as the t(11;22) of the EFTs] are readily identified ( Fig. 8-15), and (importantly) new translocations or deletions will also be discovered, which ultimately lead to discovery of new translocations, variants, or even new genes involved in the etiology of the tumor. The negative side of cytogenetics is the success rate: perhaps only 50% of the time is diagnostic material cultured.[262,263] and [264] These cases are not negative; they are uninformative. Thus, a negative result from a lack of metaphase cells to karyotype is *not* a negative result. Even if metaphase cells are present, an occult translocation may be present, for example, and still be called “normal,” or a nonspecific abnormality. Thus, positive results by cytogenetics are exceedingly important; negative results cannot be counted on.

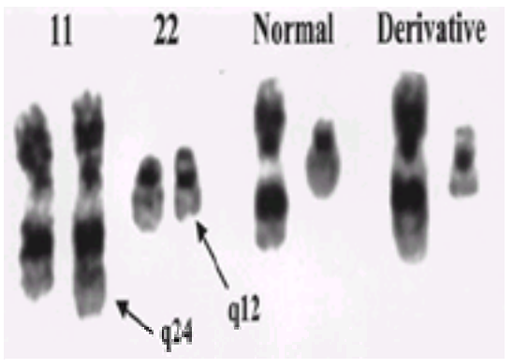


**FIGURE 8-15.** Ewing's cytogenetics. Tumor cytogenetics was the first purely genetic diagnostic analysis of tumors. A classic example, Ewing's sarcoma, routinely shows a reciprocal translocation of the long arms of chromosomes 11 and 22 [t(11;22)], resulting in the formation of derivative chromosomes 11 and 22 paired with a normal 11 and 22, as seen here. This was the first clue leading to the later identification of a specific chimeric gene in this disease.

### Fluorescent *In Situ* Hybridization

These limitations of traditional cytogenetics spawned an approach, that of interphase FISH, that requires no metaphases but which can yield exceedingly rich information from simple specimens such as frozen tissue, touch preparations, and even ordinary paraffin-embedded sections ( Fig. 8-16).[265,266,267,268,269,270,271,272,273,274,275,276] and [277] New translocations or genes cannot be found by this technique (by definition, one uses fluorescently labeled DNA probes to detect a translocation, for example). Nonetheless, the speed, specificity, and utility of this method have made it an important diagnostic tool in short order.[277,278]

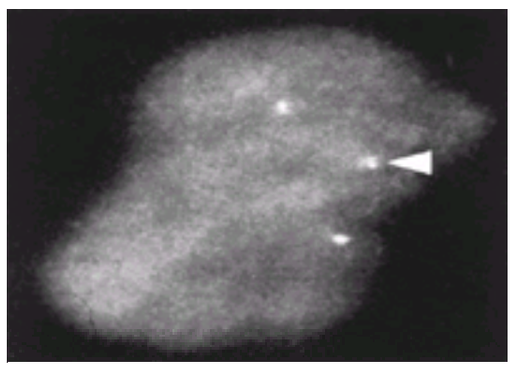

**FIGURE 8-16.** Ewing's fluorescent *in situ* hybridization (FISH). Chimeric genes, such as the EWS-FLI1 responsible for Ewing's sarcoma, are easily identified by FISH, now in routine diagnostic use, as illustrated here. Three signals are identified compared to the normal four due to the fusion of one copy of EWS and one copy of FLI1, resulting in a compound single signal (*arrowhead*), accompanied by one signal each from normal EWS and FLI1. (Courtesy of D. Lopez-Terrada, Texas Children's Hospital.)

The predominant use of FISH to date has been to detect genomic alterations, but it can also be used to detect gene expression by hybridization with mRNA in touch preparations or tissue sections. This method is less sensitive than PCR and requires better RNA preservation, and is therefore not in widespread use for diagnostic purposes, although it is an important research tool. [279]

Diagnostic use of FISH has really only begun. Refinements with new, more easily detected probes applicable to tissue sections, combined with routine *in situ* amplification of target, perhaps enhanced by secondary detection methods, such as those applied to immunohistochemistry (e.g., avidin-biotin complexes) to detect even normal gene expression in addition to gene translocations, amplification, or deletion, will likely make this technique as important as immunohistochemistry in the future.

### Spectral Karyotyping

The development of methods to label and detect individual chromosomes using spectral analysis of emitted fluorescence, thereby ascribing computer-generated false colors to individual chromosomes, is a major advance in the analysis of chromosomes, especially because metaphase and even interphase chromosomes can be visualized.[280,281,282,283] and [284] This is particularly important given the frequent failure of conventional cytogenetics to culture tumor cells, thereby obviating any chance of detecting constitutional chromosomal abnormalities (e.g., number, translocation, and deletions) as well as those tumors in which successful culture of tumor cells has uniformly failed (e.g., alveolar soft part sarcoma). [285,286]

Despite the relative youth of this technology, it is rapidly being deployed to analyze a host of tumors heretofore difficult to analyze. Cryptic inversions, translocations, numerical abnormalities, and similar chromosomal abnormalities can now be readily identified, and are, in a host of childhood (and adult) tumors, including lymphoma, NB, and RMS.[286,287,288,289] and [290] There is a very real possibility that this method will become the benchmark method for chromosomal analysis of tumors given its enormous scope of application and sensitivity to even minute abnormalities, especially when used in conjunction with derivative techniques such as locus FISH. [286]

### Comparative Genomic Hybridization

In contrast to SKY, in which intact, fresh cells or tissue are necessary for analysis of individual chromosomes, CGH (as the name suggests) can be used to detect genomic gains or losses on a comparative basis (Fig. 8-17), using a 50/50 mix of normal control and tumor differentially fluorescently (e.g., red/green) labeled DNA hybridized to normal metaphase preparations. Anything found in excess in the tumor will fluoresce more brightly with that fluor; anything lacking will fluoresce differentially with the control fluor.[291] Not surprisingly, there has been great enthusiasm in applying this method to the detection of occult amplification, deletion, and even translocation in a large variety of pediatric tumors. [292,293,294,295,296,297,298,299,300,301] and [302]

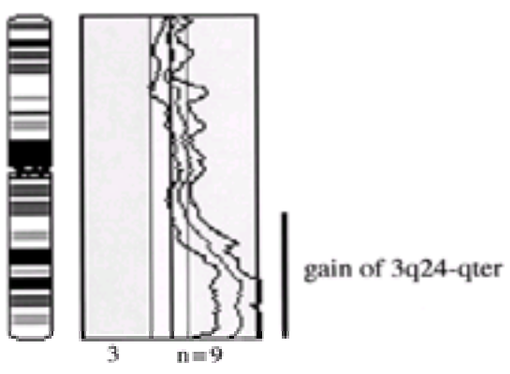


**FIGURE 8-17.** Comparative genomic hybridization analysis of chromosome 3 in malignant fibrous histiocytoma (MFH). The banding pattern of human chromosome 3 is shown on the left, and the relative hybridization levels of tumor DNA (representing nine MFH cases) versus normal DNA is shown on the right. The deviation of the

curves from the axis starting at chromosome 3q24 represents gain of genomic DNA in the MFH samples at 3q24 to the 3q terminal region ( *black bar*).

Despite the power of this method, there remains the problem that it can identify abnormalities by region, but confirmation of identity requires alternative methods. Despite this limitation, a number of novel, specific molecular defects in a variety of tumors have been identified by this method. [302,303,304,305,306,307,308] and [309] Furthermore, continued refinements of this method, such as array CGH, are generating more precise information, eventually perhaps at the single gene or locus level. [310]

### Implications for Tumor Molecular Genetics

The variety of molecular methods currently being applied or in development in cancer biology is unprecedented. Given the generally accepted truth that cancer is after all a genetic disease, this approach will undoubtedly yield a vast amount of currently unknown information about the etiology and mechanisms of cancer, including, one day, identification of fundamental mechanisms of oncogenesis within individual tumors. In the meantime, more established methods, such as PCR and correlative FISH with tumor-specific probes, are the mainstay of molecular genetic diagnosis.

## MOLECULAR GENETIC ANALYSIS OF CHILDHOOD CANCER

The identification of genetic alterations in human neoplasia is not only contributing profound insights into the oncogenic process, but it has been instrumental in the emergence of molecular diagnostics as a distinct field of pathology. Detection of tumor markers in pathologic specimens is gradually being incorporated into the diagnostic workup of tumor cases. This is necessitating changes in the way that pathologists handle tumor specimens in the surgical pathology suite to optimize the amount of information that can be derived at the molecular level. Moreover, as we begin to appreciate the specificity of these tumor markers for individual tumor types, we are forced to reevaluate the way we classify human tumors. This is particularly the case for bone and soft tissue tumors of childhood, which tend to be extremely primitive in appearance and therefore very difficult to differentiate from each other on morphologic grounds alone. [311] In fact, the detection of tumor-specific chromosomal translocations and gene amplifications in pediatric solid tumors is playing an increasingly important role in the diagnostic and prognostic stratification of these lesions. The following is a brief review of the molecular genetics of pediatric solid tumors, followed by a discussion of molecular tests currently used in the diagnostic workup of these tumors and recommendations for optimal processing of tumor tissue for such studies.

### Differential Diagnosis of Primitive Bone and Soft Tissue Solid Tumors of Childhood

Whereas organ-specific tumors of childhood, such as Wilms' tumor, hepatoblastoma, and pancreatoblastoma, are often less of a diagnostic dilemma, primitive pediatric solid tumors with a more diverse distribution remain difficult to diagnose at the time of specimen acquisition. In particular, pathologic classification in any given case of pediatric bone or soft tissue sarcoma continues to pose a frequent challenge for the surgical pathologist despite considerable histological, immunohistochemical, and ultrastructural literature on this topic. [311,312] This is not an insignificant issue because initial diagnosis often determines which treatment protocol a patient is entered on, and is therefore a critical prognostic factor. The group of malignancies known as the SRCTs are still formidable diagnostic problems, as described under Small Round Cell Tumors of Childhood, due to the relative lack of differentiation in these tumors. [311,312] Traditionally included in this group are the EFTs of peripheral primitive neuroectodermal tumors (formerly pPNETs), ARMS and ERMS, and NB. Other entities also entering this differential diagnosis include intraabdominal DSRCT (IDSRCT) and malignant lymphoma, particularly ALCL in the pediatric population ( Table 8-1). Among fibrous or spindle cell malignancies, SS, congenital or infantile fibrosarcoma (CFS), adult-type fibrosarcoma (ATFS), MPNST, and malignant fibrous histiocytoma must be considered. [311] The issue is further complicated by the fact that spindle cell lesions such as SS can sometimes have a small round cell cytologic appearance. [313]

### Molecular Genetics of Pediatric Solid Tumors

Molecular studies have contributed to an expanding list of genetic abnormalities in pediatric solid tumors, including chromosomal translocations and inversions; amplification of proto-oncogenes involved in cell growth and differentiation; loss of tumor suppressor genes by mutation DNA methylation, or deletion; abnormalities of genomic imprinting; alterations in DNA repair mechanisms; telomerase activity; and other abnormalities. From a clinicopathologic perspective, however, it only makes practical sense for a diagnostic molecular pathology laboratory to screen for those recurrent genetic changes that have been rigorously correlated with specific pathologic or clinical subgroups. The following discussion, therefore, focuses only on genetic abnormalities with well-established diagnostic or prognostic relevance, including chromosomal translocations leading to tumor-specific gene fusions in pediatric sarcomas, and gene amplifications and other genomic alterations in NB (Table 8-2). It remains to be determined whether some of the other alterations listed earlier in this section will eventually also show clinicopathologic correlations useful in clinical practice.

#### *Gene Fusions in Pediatric Solid Tumors*

Cytogenetic studies of several childhood sarcomas have identified reciprocal chromosomal translocations that are correlated with specific tumor types. Molecular cloning of the translocation breakpoints have identified in-frame fusions between genes located at the breakpoints of each partner chromosome; these gene fusions result in the expression of chimeric oncoproteins that appear to function in transformation by dysregulating gene transcription or altering cellular signal transduction pathways. [31,314] Known gene fusions in pediatric solid tumors are now reviewed.

#### *EWS-ETS* Gene Fusions in the Ewing's Tumor Family.

The diagnosis of Ewing's tumor and other EFTs, which can occur in bone or soft tissues, has traditionally depended on clinical features along with demonstrable intracellular glycogen accumulation, variable evidence of neural differentiation, and staining for the MIC2 antigen using the O13 antibody (reviewed elsewhere [312]). Aside from the latter, however, none of these features is particularly specific. In fact, even MIC2 staining, although present in greater than 95% of EFTs, [142,147] has been described in other tumors such as RMS, NB, and lymphoblastic lymphoma. [149,315] One feature of EFTs that does appear to be consistent is that they share common genetic rearrangements, with approximately 85% of cases showing a t(11;22)(q24;q12) chromosomal translocation ( Fig. 8-15). [316] Molecular cloning of the translocation breakpoint has identified an in-frame fusion of the *EWS* gene from chromosome 22q12 with *FLI1* from chromosome 11q24, a member of the *ETS* family of transcription factors ( Fig. 8-18A). [317,318] An additional 10% to 15% of cases carry a variant t(21;22)(q22;q12) translocation in which *EWS* is fused to another *ETS* gene, *ERG* from chromosome 21q22. [319] More rarely (likely in 1% or less of tumors), *EWS-FEV*, *EWS-EVI1*, and *EWS-ETV1* gene fusions resulting from t(2;22), t(7;22) and t(17;22) translocations, respectively, have been reported. [320,321] and [322] It is now thought that virtually all EFTs carry some form of *EWS-ETS* gene fusion, and that these rearrangements are pathognomonic of the EFTs. [323]

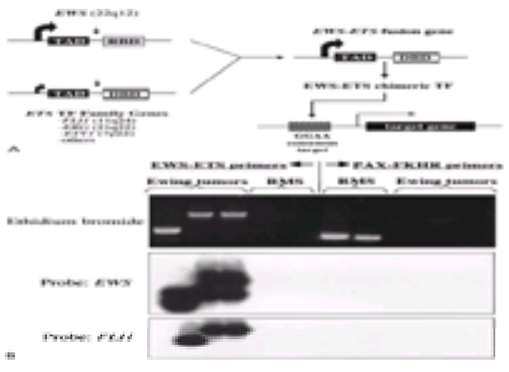


**FIGURE 8-18. A:** Schematic diagram of *EWS-ETS* gene fusions in Ewing's family tumors. As a result of chromosomal translocation breakpoints (*small downward arrow*), the *EWS* gene on chromosome 22q12 becomes fused to one of several ETS family genes, most often *FLI1* on 11q24 or *ERG* on 21q22. This fuses the transcriptional activation domain (TAD) of EWS with the DNA-binding domain (DBD) of the respective *ETS* gene and places the resulting *EWS-ETS* fusion gene under the control of the strong *EWS* promoter. This rearrangement replaces the RNA-binding domain (RBD) of EWS with the ETS DBD, creating a chimeric transcription factor (TF) that binds to ETS consensus GGAA sites on DNA and activates transcription of ETS target genes thought to be involved in

EWS-ETS–induced transformation. **B:** Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of Ewing's family tumors. Consensus primers for *EWS-ETS* fusion transcripts are used in RT-PCR experiments (*left side*) to demonstrate *EWS-FLI1* transcripts in the three Ewing's tumor samples but not in the two rhabdomyosarcoma (RMS) samples by ethidium bromide staining of agarose gels (*top panel*). This is confirmed by probing the amplification products with oligonucleotide probes for *EWS* (*middle panel*) and *FLI1* (*bottom panel*). On the right side, primers for the *PAX-FKHR* gene fusions of alveolar rhabdomyosarcoma are found in the RMS cases but not in the Ewing's tumor samples.

Expressed *EWS-ETS* chimeric proteins are oncogenic in NIH3T3 cells[318] and appear to function as aberrant transcription factors binding to ETS consensus sequences of target genes (Fig. 8-18A).[318,324,325,326 and 327] A number of potentially interesting target genes of EWS-ETS–mediated transcriptional activation have been described,[328,329,330 and 331] including stromelysin 1, cytochrome P-450 F1, cytokeratin 15, manic fringe, and E2-C, a cyclin-selective ubiquitin-conjugating enzyme involved in degradation of cyclin B. How these putative EWS-ETS targets are involved in oncogenesis remains unknown. An interesting possible role for EWS-ETS chimeric oncoproteins is suggested by the recent observation that EWS-FLI1 and other EWS-ETS proteins down-regulate expression of the TGF-b type II receptor TGF-b RII), a putative tumor suppressor gene.[332,333] TGF-b signaling through this receptor induces apoptosis in many cell types, and therefore repression of TGF-b RII expression may provide Ewing's tumor cells with a mechanism to elude a major pathway leading to programmed cell death. Another intriguing finding is that inactivation of the *p16* INK4a locus occurs frequently in EFTs, suggesting that loss of the pRb pathway may be important in Ewing's tumor oncogenesis.[334] These tumors also appear to use a number of autocrine growth factor pathways. Insulin-like growth factor-1 (IGF-1) is expressed by EFTs,[335] and oncogenicity of EWS-FLI1 requires the presence of an intact IGF-1 receptor pathway.[336] Moreover, Ewing's tumor cells have been found to express neural peptides such as the bombesin homolog gastrin-releasing peptide,[337] although the role of gastrin-releasing peptide in tumorigenesis remains unclear.

### Other *EWS*-Associated Gene Fusions in Small Round Cell Tumors of Childhood

IDSRCT is a recently described aggressive malignancy in late adolescent males that coexpresses skeletal muscle, neural, and epithelial antigens.[312] This tumor most commonly occurs in association with serosal surfaces, and as such can be confused with extraosseous pPNETs. A characteristic t(11;22)(p13;q12) translocation in IDSRCT fuses *EWS* with the *WT1* tumor suppressor gene from 11p13.[29,338] Another *EWS*-associated gene results from the t(12;22)(q13;q12) translocation of malignant melanoma of soft parts,[339] in which *EWS-ATF1* gene fusion transcripts are expressed in tumor cells. This melanin-producing tumor, also known as clear cell sarcoma of tendons and aponeuroses, mostly occurs in limbs of young adults.[312] The epithelioid appearance of tumor cells can sometimes cause confusion with SRCTs. Both EWS-WT1 and EWS-ATF1 chimeric products appear to be DNA-binding proteins and likely function as aberrant transcription factors.

### *PAX-FKHR* Gene Fusions in Alveolar Rhabdomyosarcoma

Childhood RMS is generally subdivided into ERMS (approximately 65%) and more primitive forms including ARMS (approximately 20%), and so-called undifferentiated sarcoma (approximately 15%).[340] The diagnosis of RMS is based on morphology and demonstration of myogenic differentiation in tumor cells (reviewed elsewhere[312]), including desmin and MSA immunostaining and expression of the MyoD family of myogenic transcription factors.[341,342] However, these markers can be expressed in other tumor types.[311,343,344] Of RMS subtypes, only ARMS is characterized by a diagnostic rearrangement. Approximately 60% of cases demonstrate a t(2;13)(q35;q14) translocation, which fuses the *PAX3* gene from 2q35 with the *FKHR* gene from 13q14; a smaller proportion of approximately 15% to 20% have t(1;13)(p36;q14) translocations that fuse *PAX7* from 1p36 with *FKHR*.[345,346] *PAX3* and *PAX7* are members of the PAX family of transcription factors, whereas FKHR is a member of the forkhead family of developmentally regulated transcription factors.[347] Resulting *PAX3-FKHR* and *PAX7-FKHR* fusion transcripts can be detected in tumor tissue by RT-PCR (Fig. 8-18B).[345,346] Although several molecular studies indicate that *PAX3-FKHR* and *PAX7-FKHR* gene fusions are present in greater than 85% of ARMS cases,[20,29] others have reported that significantly higher percentages of ARMS cases lack these fusions,[348] indicating that a proportion of ARMS cases have molecular alterations that are yet to be defined. In studies by Kelly et al.[349] comparing clinical features of ARMS cases with either *PAX3-FKHR* or *PAX7-FKHR* gene fusions, it was reported that *PAX7-FKHR* rearrangements are associated with younger age and extremity disease, and in these studies there was a trend toward improved overall survival in the *PAX7-FKHR* group. These studies have been extended to a larger series of ARMS cases as will be discussed below.

### *NPM-ALK* Gene Fusion in Anaplastic Large Cell Lymphoma

ALCL, also called *Ki-1 lymphoma*, is a distinct clinicopathologic subtype of intermediate-grade non-Hodgkin's lymphoma that can occur in children (see also Chapter 24)[350] ALCL has a wide range of morphologic appearances, although it is most often described as having large pleomorphic cells expressing the CD30 (Ki-1) antigen. An as-yet undetermined proportion of ALCL cases have a t(2;5)(p23;q35) translocation, which has recently been cloned.[351] A gene fusion is formed that encodes the N-terminal portion of a nonribosomal nucleolar phosphoprotein, nucleophosmin (NPM), fused to the tyrosine kinase domain of a novel transmembrane tyrosine-specific protein kinase, anaplastic lymphoma kinase (ALK).[351] A variant *NPM-ALK* fusion has also been described.[352] The incidence of the *NPM-ALK* gene fusion in ALCL is somewhat controversial and remains under investigation. The situation is complicated by the fact that ALCL as originally described appears to be a heterogeneous disease morphologically, cytogenetically, and clinically. This is reflected in the literature by a wide range (12% to 100%) in the incidence of this gene fusion in ALCL (reviewed elsewhere[353]). Current estimates are that approximately 70% of cases overall and almost 90% of childhood cases of ALCL express this gene fusion.[353,354] Neither anaplastic morphology nor the expression of CD30 appears to accurately predict the presence of this molecular genetic subtype of lymphoma. Some authors contend that immunostaining for the p80 NPM-ALK chimeric protein is a reliable diagnostic method for identifying cases with the t(2;5)(p23;q35) translocation.[354] Recently, several variant translocations in which ALK is fused to other partners have been reported.[355,356] The incidence of these rearrangements in ALCL remains to be determined.

### Gene Fusions in Childhood Spindle Cell Tumors

Gene fusions associated with pediatric solid tumors have mainly been identified and characterized in lesions with SRCT morphology. Tumor-specific translocations and resulting gene fusions have also been reported in several spindle cell tumors occurring in childhood, however—namely SS, CFS, and congenital mesoblastic nephroma (CMN). SS is a malignant neoplasm of children and young adults showing variable combinations of spindle cell and epithelial components. The latter stains positively for cytokeratin and EMA,[312] causing diagnostic confusion with epithelial tumors if SS is not suspected. A specific t(X;18)(p11.2;q11.2) translocation is present in greater than 90% of either biphasic or monophasic forms of SS (Fig. 8-19A).[357] Molecular cloning of the translocation breakpoints actually identified two different rearrangements—namely the fusion of the *SYT* gene from 18q11.2 with either of two closely mapped Xp11.2 genes, *SSX1* or *SSX2* (Fig. 8-19B).[358,359] It has been suggested that the *SYT-SSX2* gene fusion is associated with a monophasic phenotype,[360] but this has not been confirmed in other studies.[359] *SYT-SSX1* or *SYT-SSX2* fusion transcripts can be detected in more than 90% of SS,[359,361] and therefore assays to screen for these fusions provide a sensitive tool for the diagnosis of SS.

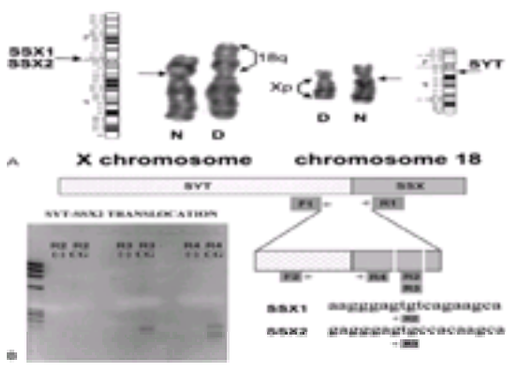


**FIGURE 8-19.** Synoviosarcoma cytogenetics and molecular genetics. **A:** Synoviosarcoma fusion cytogenetics. In this disease, the short arm of the X chromosome near the centromere (at a gene, SSX 1 or 2) is translocated to the near centromeric long arm of chromosome 18 (at another gene, SYT), as illustrated by the arrows labeled 18q and Xp, respectively. This results in the formation of derivative chromosomes X and 18 and a fusion gene, SYT-SSX, paired with a normal chromosome X and 18, as also labeled here. **B:** Fusion gene detection using polymerase chain reaction (PCR). Two common break points occur on the X chromosome, resulting in

the creation of two forms of the chimeric gene, identified as SSX1 and SSX2, respectively. Both can be amplified with universal PCR primers, labeled F1 and R1 here, which amplify sequence flanking the break point. This can be specifically amplified using sequence-specific primers, as illustrated here (R2 and R3 for SSX1 and SSX2, respectively). In the case of paraffin-embedded material in which messenger RNA is highly fragmented, the resulting specific amplified signal can be further amplified by nested PCR (here illustrated using another primer pair, F2 and R4), resulting in detection that might not otherwise be possible, as illustrated in the accompanying PCR gel. (Courtesy of Deborah Schofield, Children's Hospital, Los Angeles.)

CFS is a cellular spindle cell tumor of the soft tissues that generally presents before age 2 years. [362] As the name implies, many cases are congenital. Although CFS shows frankly malignant cytology and a high recurrence rate, it has a very good prognosis with an 80% to 90% overall survival and only a 10% metastatic rate. [362] It must therefore be differentiated from so-called ATFS of older children, which appears to have poor prognosis similar to fibrosarcoma occurring in adults. [363] Knezevich et al.[364] recently identified a t(12;15)(p13;q25) translocation in CFS that fuses the *ETV6* (*TEL*) gene from 12p13 with the 15q25 neurotrophin-3 receptor gene, *NTRK3* (*TRKC*) (Fig. 8-20A). *ETV6-NTRK3* fusion transcripts were not present in ATFS and appear to be specific for CFS among childhood soft tissue tumors ( Fig. 8-20B). The predicted chimeric product contains the helix-loop-helix (HLH) dimerization domain of ETV6 fused to the protein tyrosine kinase (PTK) domain of NTRK3. ETV6-NTRK3 has potent transforming activity in NIH3T3 cells, which requires both the HLH and PTK domains of the fusion protein. [365] Homodimerization through the HLH domain leads to activation of the NTRK3 PTK domain, which appears to dysregulate NTRK3 signal transduction pathways in tumor cells. Several groups [364,366,367] have recently demonstrated identical *ETV6-NTRK3* gene fusions in another pediatric solid tumor, CMN. CMN is an infantile spindle cell tumor of the kidney that is subdivided into “classical,” “mixed,” and “cellular” forms based on the degree of cellularity and mitotic activity. The histogenesis of CMN remains obscure, but a relationship between cellular CMN and CFS has been postulated based on morphologic and ultrastructural similarities. [368] This is supported by similarities in clinical behavior: whereas both classical and cellular forms of CMN occur in very young children and are generally thought to have an excellent prognosis, reports of local recurrences and metastatic spread are almost exclusively associated with the cellular variant. [369,370,371] and [372] To test this relationship at a molecular level, Knezevich et al.[373] screened CMN cases for *ETV6-NTRK3* gene fusions: eight of nine cellular CMNs and two of two mixed CMNs were fusion positive but all four classical CMNs tested were negative (Fig. 8-20B). Other studies did not find these transcripts in the mixed form. [366] Taken together, these studies indicate that classical and cellular CMN have different genetic features, and support the concept that cellular CMN is histogenetically related to CFS. Virtually all CFS and cellular CMN cases with the *ETV6-NTRK3* gene fusion were found to carry an extra copy of chromosome 11 (Fig. 8-21).[367,373] Trisomy 11 has been previously described for these lesions. [374,375,376] and [377] Although there are numerous explanations as to why tumor cells might select for both the *ETV6-NTRK3* gene fusion and trisomy 11, one intriguing possibility is that this provides cells with an additional copy of the insulin-like growth factor 2 gene ( *IGF-2*), which localized to chromosome 11p15.5 and is known to induce anti-apoptotic signaling by binding to the IGF-1 receptor. [378]

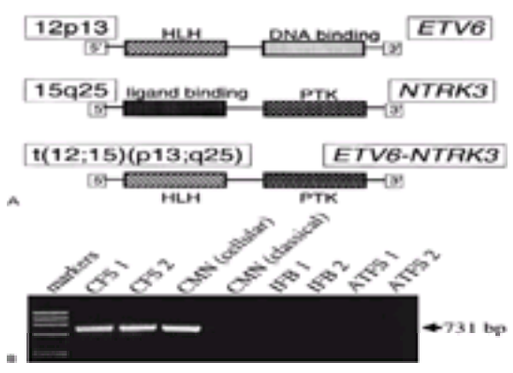


**FIGURE 8-20. A:** Schematic diagram of *ETV6-NTRK3* gene fusions in congenital fibrosarcoma (CFS) and cellular congenital mesoblastic nephroma (CMN). As a result of t(12;15)(p13;q25) translocations, exons encoding the helix-loop-helix (HLH) dimerization domain of the 12p13 *ETV6* (*TEL*) transcription factor gene are fused to exons encoding the protein tyrosine kinase (PTK) domain of the *NTRK3* (*TRKC*) neurotrophin-3 receptor gene on 15q25. This results in expression of a chimeric tyrosine kinase that undergoes ligand-independent dimerization and PTK activation. **B:** Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of the *ETV6-NTRK3* gene fusion in CFS and CMN. With primers from 5' sequences of the *ETV6* gene and 3' sequences of the *NTRK3* gene, RT-PCR is used to amplify a 731-bp amplification product in CFS and cellular CMN, but not in classical CMN, infantile fibromatosis, or adult-type fibrosarcoma.

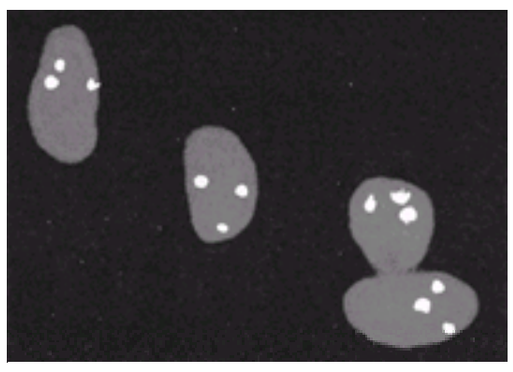

**FIGURE 8-21.** Trisomy 11 in congenital fibrosarcoma and cellular congenital mesoblastic nephroma (CMN). Four interphase cells from a case of cellular CMN are shown after probing with a centromeric probe for human chromosome 11. Three copies of chromosome 11 are demonstrated for each cell.

### *Molecular Genetic Alterations in Neuroblastoma*

The diagnosis of NB is generally less problematic than that of other SRCTs given the usual occurrence of a catecholamine-secreting tumor arising in the adrenal gland of a young child and the distinctive neural phenotype of virtually all cases. [311] The marked variability in clinical behavior of this tumor, however, ranging from spontaneous regression to metastatic growth and early death, has prompted an intense search for reliable predictors of prognosis in NB. A number of genetic abnormalities have been identified in NB that correlate to varying degrees with clinical outcome, including *MYCN* amplification, deletion of distal chromosome 1p, deviations from diploid DNA content, expression of neurotrophin receptors (particularly TRKA), amplification of 17q, and detection of telomerase activity (reviewed elsewhere[30,379]). Of these, *MYCN* amplification (Fig. 8-9) and 1p deletions—each present in approximately 30% to 50% of NB primary tumors—have in recent years been regarded as the best predictors of poor prognosis. A strong correlation has been found between these two prognostic markers. [380] Brodeur et al.[379] have suggested that NB should be classified into three distinct subsets based on biologic and clinical features. The first includes hyperdiploid tumors that express TRKA, do not overexpress *MYCN*, and are low-stage, favorable-prognosis tumors occurring in very young children. The second group is made up of near-diploid, non- *MYCN* amplified tumors with 1p deletion and low TRKA expression; they tend to present as higher-stage lesions in older children and have an intermediate outcome. The third group includes *MYCN*-amplified, near-diploid tumors with 1p deletions and low or absent *TRKA* expression; these tumors present as advanced-stage disease in older children and have a very poor prognosis. This is summarized for *TRKA* and *MYCN* in Figure 8-10. Further studies are necessary to confirm the clinical utility of this classification scheme, but it is proposed that the three different subsets represent genetically distinct forms of NB. [379] More recently, it has become apparent that gains of chromosome 17q21-qter may be the most frequent cytogenetic abnormality (greater than 50%) in NB. [381,382] and [383] This abnormality has been associated with advanced disease, age older than 1 year, deletion of chromosome arm 1p, and amplification of the *MYCN* oncogene, all of which have previously been associated with an adverse outcome. In fact, Bown et al.[381] have recently shown that gain of this region was the most powerful prognostic factor in multivariate analysis of a series of NB patients, strongly indicating that gain of 17q is an important prognostic factor in children with NB. As discussed below, we currently limit our analysis to screening for *MYCN* amplification and deletions of 1p in primary NB cases, although we are considering adding 17 gains to the analysis of NB.

## Molecular Genetic Tests for Pediatric Solid Tumor Diagnosis

Detection of the discussed tumor markers in pathologic specimens holds great promise as a diagnostic modality and, in fact, molecular genetic testing is already standard practice in many centers. However, it is important to note that these techniques place new requirements on the initial handling of specimens arriving in the surgical pathology suite. It cannot be emphasized enough that for many of the molecular assays described below, the most critical factor for success is the proper initial tissue processing. The following is a summary of the tests we routinely perform at the Children's and Women's Health Center of British Columbia and a discussion of some practical issues for the optimization of these tests.

### *Molecular Assays for Pediatric Solid Tumor Gene Fusions*

The development of RT-PCR assays to detect specific gene fusions now offers a sensitive and reliable diagnostic modality for the identification of SRCTs and other pediatric solid tumors.[29,31,359,384,385] These assays have been applied to the detection of minimal residual disease in pediatric patients diagnosed with solid tumors.[386,387,388 and 389] This specific application consists of the screening of bone marrow or peripheral blood samples from patients with previously identified gene fusions as a means for detecting residual tumor cells in these specimens. This is increasingly requested by oncologists at our institutions both for staging purposes and for evaluation of remission before bone marrow transplantation. We have also tested autologous bone marrow samples to screen for the presence of tumor cells before transplantation. The specific tests we perform and references for the methods are included in Table 8-4, but a number of general points can be made that apply to all the gene fusion assays.

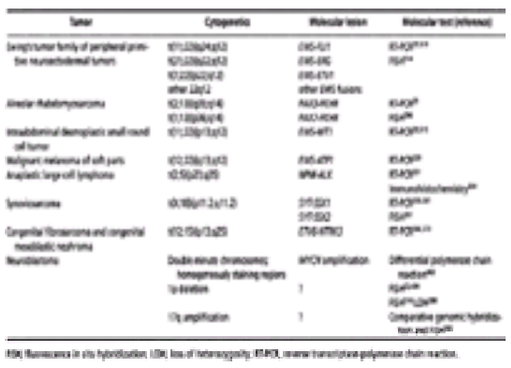

**TABLE 8-4. MOLECULAR TESTS USED IN THE DIAGNOSTIC WORKUP OF PEDIATRIC SOLID TUMORS**

### Reverse Transcriptase-Polymerase Chain Reaction from Frozen Tissue

Each RT-PCR assay depends on the successful isolation of RNA from representative tumor tissue. Far and away the best source of intact RNA is snap-frozen tissue stored at –70$^{o}$C or colder, as many of the commonly used fixatives result in appreciable RNA degradation and unacceptably low yields. Tissue should be frozen as soon as possible after surgery or biopsy to minimize autolysis. It is also essential that the presence of viable tumor and minimal necrosis is present in the tissue to be analyzed. We therefore routinely snap-freeze tissue fragments in optimal cryopreservation temperature medium, allowing us to easily cut a frozen section on a cryostat for H&E staining before further processing of the sample.[385] We find that total RNA isolated using the acid guanidinium-phenol/chloroform method of Chomczynski and Sacchi[390] or a related method provides adequate RNA quality. A number of commercially available reagent kits such as Trizol (Gibco/BRL) are very useful for RNA isolation from primary tumor tissue. At least 100 to 200 mg of primary tumor tissue is required, which corresponds to five to ten 5-µm sections (depending on the size of the tissue block) cut on a cryostat equipped with clean cryostat blades.[385] These sections should be placed directly into denaturing solutions such as solution D.[390] RNA is subsequently converted to cDNA using random primers. The resulting cDNA mixture is then subjected to PCR using primer pairs from coding sequences of each of the involved partner genes. An example of this process is shown for detection of *ETV6-NTRK3* fusion transcripts of CFS and CMN in Figure 8-20B. These assays have been optimized to maximize specificity and to minimize the number of false-positives (reviewed elsewhere [29]). Positive controls, usually consisting of cell lines expressing the gene fusion in question, are always included, as are negative controls lacking these hybrid genes. Alternatively, previously tested positive primary tumors can be used, but this of course may quickly deplete sample material. In extenuating circumstances, amplified PCR fragments from specific assays can be cloned into and used as positive controls,[385] but this is not recommended, as cloned products will be readily amplified even if assay conditions are suboptimal for primary tumor tissue. PCR fragments are separated by gel electrophoresis using 2% agarose gels and visualized by ethidium bromide staining. For confirmation of identity, PCR products are blotted onto nylon filters and probed with labeled oligonucleotides specific for each gene fusion.[29]

Several pediatric solid tumors are characterized by more than one variant of a distinct family of gene fusions, making the use of consensus primers that amplify multiple fusion types within each family particularly useful. We therefore use consensus primers that will amplify both *EWS-FLI1* and *EWS-ERG* gene fusions in EFTs, and use *FLI1*- or *ERG*-specific oligonucleotides as probes to distinguish between these fusion types.[29,319] An example of this process is shown in Figure 8-18B. A similar strategy is used to differentiate *PAX3-FKHR* and *PAX7-FKHR* gene fusion in ARMS,[29] and between *SYT-SSX1* and *SYT-SSX2* gene fusions in SS.[361] Some authors have advocated the use of multiplex RT-PCR assays that amplify more than one family of gene fusions simultaneously.[20]

In terms of sensitivity, *EWS-ETS* gene fusions are thought to be present in virtually all EFTs.[323] Similarly, *ETV6-NTRK3* transcripts appear to be expressed in at least a large majority of CFS and cellular CMN, as is reported for *EWS-WT1* and *EWS-ATF1*, as well as for *SYT-SSX1* and *-SSX2* gene fusions in IDSRCT, malignant melanoma of soft parts, and SS cases, respectively. These assays can therefore be regarded as true diagnostic tests for each tumor subclasses. As mentioned above, however, in our clinical experience it appears that as many as 20% to 30% of cases with ARMS phenotype lack detectable *PAX3-FKHR* or *PAX7-FKHR* gene fusions.[391] Therefore, although these rearrangements are specific for ARMS, they may not be expressed in all cases of suspected ARMS, as this tumor subclass may be more heterogeneous than previously appreciated. The same can be said for ALCL, in which *NPM-ALK* gene fusions do not appear to be present in all cases analyzed, and the incidence of variant gene fusions remains to be determined.

### Reverse Transcriptase-Polymerase Chain Reaction from Archival Tissue

It is well known that fixation of tissue and embedding in paraffin results in significant RNA degradation, which has previously mitigated against the use of archival tissue in RT-PCR assays. However, recent improvements in the recovery of mRNA from paraffin-embedded tissue have resulted in a reevaluation of this application. A number of reports have described the detection of SRCT- or spindle cell–associated gene fusions using formalin-fixed, paraffin-embedded tissue.[366, 391a,391b,391c and 392] In our own studies, several general factors appeared to be critical for successful recovery of amplifiable RNA amenable to RT-PCR analysis (unpublished results). First, alcohol fixation gave the best results followed by formalin; Bouin's and B5 solutions did not yield usable nucleic acid. Second, minimizing the time of fixation to less than 24 hours generally gave better results. Third, optimal results were obtained when the size of the expected amplification products was reduced to less than 200 bp, as has been reported by others.[366, 391a,391b,391c and 392] Fourth, it was often necessary to hybridize the PCR products with specific oligonucleotide probes to detect positive reactions. Our current success rate is approximately 60% to 70% in terms of deriving amplifiable RNA from paraffin-embedded tissue, as assessed using RT-PCR assays for the phosphoglycerate kinase gene (*PGK*).[392] For the specific gene fusion assays, the relative success rates depend on the specific assays, with RT-PCR detection of *EWS-ETS*, *EWS-WT1*, *SYT-SSX*, and *ETV6-NTRK3* being quite robust in paraffin-embedded tissue. In contrast, detection of *PAX-FKHR* gene fusions in paraffin-embedded tissue from ARMS cases is generally less successful and, at least in our institutions, remains an experimental tool. Clearly the optimization of this approach will greatly facilitate the application of RT-PCR to the diagnosis of pediatric solid tumors, and further studies are warranted in this regard.

### Gene Fusion Detection Using Fluorescence *In Situ* Hybridization

The cloning of fusion gene partners and subsequent availability of FISH probes for those genes have made FISH a natural alternative method to study the genetics of solid tumors.[393,394 and 395] Several strategies are currently used to screen for solid tumor gene fusions: (a) one-color FISH with a probe or probe cocktail that spans one of the fusion partners; probing would then be expected to show three signals in tumor cells (including two derivative chromosomes and one normal copy) as opposed

to two copies in normal cells; (b) two-color FISH in which flanking probes from each side of one of the partner genes are labeled with different fluors; normal cells will demonstrate two overlapping or fusion of the signals, whereas tumor cells will demonstrate splitting of the one of the signals to each derivative chromosome; and (c) two-color FISH in which probes from each of the involved genes is labeled with different fluors; in this case normal cells will show two separate signals for each fluor representing the normal loci, whereas tumor cells will show a fusion signal representing the derivative locus in addition to one separate signal for each fluor. These approaches can be used not only for metaphase cells from routine cytogenetic preparations [396] but also for interphase cells from touch preparations [314,396,397] or from paraffin-embedded sections.[22,398,399,400 and 401] In our opinion, the only major drawbacks to the application of this technique to tumor diagnosis are the limited availability of probes and the high degree of technical expertise required for successful reproducible FISH compared to RT-PCR technology.

***Molecular Testing in Neuroblastoma***

We currently screen all new NB cases for evidence of *MYCN* amplification, and we screen selected cases for deletions of distal chromosome 1p. At the time of writing, only the presence or absence of *MYCN* amplification more than ten copies, among available molecular tests, is incorporated into therapeutic stratification for NB protocols at Children's Oncology Group (the former Children's Cancer Group and Pediatric Oncology Group) institutions. [27] As discussed, several other molecular parameters may also ultimately be used in this regard.

We test for *MYCN* amplification using two approaches. The first is the technique of semiquantitative differential PCR in which genomic DNA from NB tumor samples is amplified using primer sets for *MYCN* and simultaneously for a control single copy gene such as b-globin. [402] PCR products are then resolved using agarose electrophoresis, and *MYCN* amplification is identified by visual or densitometric comparison of the *MYCN* band intensity with that of the single-copy control band ( Fig. 8-8). NB cell lines such as IMR-32,[403] with known levels of amplified *MYCN*, are run for comparison, as are normal samples that function as controls for single-copy *MYCN*. This approach is particularly useful, as the assay can be run with as little as 25 to 50 ng of DNA. Moreover, the assay works well using DNA isolated from paraffin-embedded, formalin-fixed tissue blocks. A major caveat of this technique is that if the tissue fragment being tested consists of less than 25% tumor cells, then amplification will not be readily evident. Therefore frozen sections should be stained for histology before analysis. This caveat is also the reason we use FISH as an independent approach to screen NB cases for *MYCN* amplification. We use a commercially available *MYCN* cosmid probe (Oncor, now Ventana Medical Systems, Tucson, AZ) for FISH studies performed on metaphase or interphase cells from fresh NB samples, detecting either double minute chromosomes or homogeneously staining regions containing *MYCN* amplicons.[404] This technique has been adapted to isolated nuclei from paraffin-embedded tissue. [7,23] We find an excellent correlation between the results of differential PCR and FISH studies, and we do not routinely perform Southern blot analysis for *MYCN* amplification.

Deletions of distal 1p are analyzed by two-color FISH using an a-centromeric probe for chromosome 1 in conjunction with a telomeric probe for chromosome 1p, D1Z2 (Fig. 8-21) (Oncor, now Ventana). This approach requires metaphase cells, which we obtain from routine cytogenetic cultures, if available. The caveat of such a strategy is that it may miss small interstitial deletions of 1p36 as well as point mutations in the putative tumor suppressor gene involved in NB.

**Molecular Genetic Testing from Paraffin-Embedded Tissues**

Knowledge of the types of molecular genetic tests that are available for the diagnostic workup of pediatric solid tumor samples is essential for proper handling of tumor specimens. Although FISH and genomic PCR techniques have been well adapted to paraffin-embedded archival tissues, studies requiring mRNA analysis, such as the detection of fusion transcript expression, are still problematic. Until, and if, RT-PCR can reliably be performed from fixed tissues, it remains the responsibility of the pathologist to ascertain that tissue is frozen at the time of biopsy of a pediatric solid tumor, as illustrated in Figure 8-6. This ensures that all molecular tests are possible for that case, whether performed at local institutions or in molecular pathology reference laboratories.

## MICROARRAYS, POLYMORPHISMS, AND WHOLE GENOME STUDIES

As informative as single-gene analyses have been in the study of childhood cancer, technologic advances have opened up an entirely new approach to analysis of the functional genome, a point of considerable interest to oncologists and cancer biologists alike. [405,406] Increasingly, it has become obvious that the power of this technology to elucidate the multiplicity of genomic defects inherent in oncogenesis, and to potentially identify diagnostic, prognostic, and therapeutics targets, is unprecedented. For that reason alone, it is worth examining the basic technology and assumptions in some detail, as these methods will certainly come to play an increasing role in the evaluation of human malignancy and disease (and health) in general.

Although biotechnology is an area of intense development, with nearly constant innovation and development of new methods, there are currently several well-characterized approaches to analysis of the entire genome. Methods like high-throughput direct DNA sequencing (as recently used to complete the sequence of the human genome) as applied to expressed sequence tags and batch processes for detection of many, but not all, expressed genes, such as serial analysis of genetic expression, are well known. Here, we focus on promising new developments in this area. Three particularly interesting methods are discussed:

1. Patterns of gene expression, usually identified by gene expression arrays
2. Patterns of nucleotide base changes related to gene function, usually screened genome-wide by DNA resequencing arrays
3. Patterns of gene product (e.g., protein) expression, assessed by multiple technologies ranging from mass spectroscopy to protein arrays, generically referred to as *proteomics*, to emphasize the parallels between DNA or RNA and protein assays.

The nearly universal interest in the power of these technologies to exploit the information derived from sequencing of the entire human genome has engendered an enormous development effort to create many forms of these three basic platforms. Many of these are seemingly unrelated to the current technology (all-in-one nanomachines capable of DNA, RNA, and protein analysis come to mind). None is currently well developed enough to warrant detailed discussion here. Rather, we consider the current and imminent contributions of mature technology to childhood cancer diagnosis (and ultimately treatment), noting only that the specific technology will likely diverge strikingly over the lifetime of this writing. The goal remains the same, however: a genetic explanation for the occurrence, behavior, and treatment responsiveness of a given tumor.

**Gene Expression Profiling by DNA Microarrays**

The basic technology for gene expression profiling is not new, however; it is simply enormously expanded. It is generally referred to as *microarray* or *gene chip* technology.[407,408 and 409] In essence, the same basic technique used for years to identify expressed genes on filter papers (e.g., slot blots and dot blots) has been reversed (i.e., the sample is applied to the substrate, which contains identified genes of interest) and scaled up from one or a few genes to thousands of genes, soon to encompass the entire human genome, estimated at approximately 30,000 to 40,000 discrete genes.

Two basic methods have been developed. So-called spotted microarrays use full-length cDNA clones, often expressed via phage in bacteria, cut out, and attached either covalently or via a charged moiety (e.g., poly- L-lysine) to a glass substrate (generally an ordinary glass slide with treated surface). [409] The alternative arrays use photolithography technology to synthesize short (approximately 24mer) oligonucleotides *in situ*.[407] To fairly represent genes that may be thousands of bases long, multiple oligonucleotides are chosen throughout the length of the expressed gene sequence and represented as separate “tiles” adjacent to one another. Thus, a single gene may have 20 or more short oligos represent various regions from 5' to 3' along the length of the gene. An example is illustrated in Figure 8-22. Here, each “tile” represents an oligomeric sequence chosen from exons arrayed from 5' to 3' throughout the length of the gene. To control for nonspecific hybridization, tiles with a single base mismatch are arrayed just below the matching “perfect match” tile. Hybridization intensity is compared between the two and along the length of the gene. These values are compiled and a determination of “present” or “absent” is calculated and reported, along with a quantitative value.

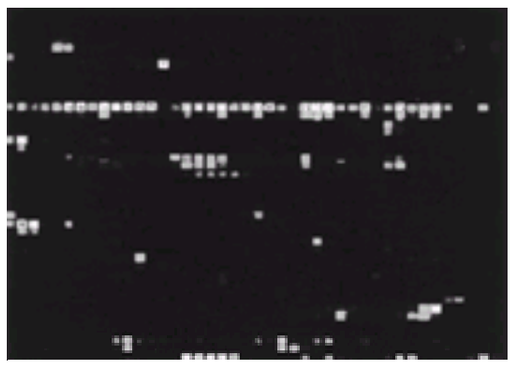

**FIGURE 8-22.** DNA gene-expression chip. DNA chips produced by the synthesis of 25-base oligomers have been popularized commercially. In these chips, a single gene is represented as a series of tiles, as seen here, each representing a 25-base stretch of the gene in question. In some versions of these chips, the genes are physically arrayed left to right, representing the gene 5' to 3' tiles, as seen here in the upper portion of the figure. When a gene is expressed at high levels, complementary RNA will hybridize and yield a bright fluorescent signal, as seen in the series of brightly fluorescent tiles. Single tiles, as seen elsewhere, are meaningless, and as is clear from this illustration, many genes are not expressed at any given moment in time.

A variant technology has also been developed, wherein spotting technology is used to spot short sequences (often approximately 50mers) on glass slides. In addition, multiple oligos, representing different regions of the gene, analogous to the photolithography arrays described above, can be spotted in adjacent spots, also often chosen from multiple stretches of the entire coding sequence of the gene of interest. [410] An example is shown in Figure 8-23. This approach combines some of the performance features of both technologies—specifically, the economy of spotted arrays with the predictable performance of short oligonucleotides as synthesized on the photolithography arrays. In addition, this method (and the photolithography arrays), unlike cDNA arrays, should enable detection of splice variants, alternate exon usage, message truncation, and related phenomena (such as chimeric genes formed from the fusion of two normal genes as a result of chromosomal translocation, as discussed in the section Gene Fusions in Pediatric Solid Tumors). This presumes, of course, that the oligos chosen are a fair representation of exon-by-exon sequences within the gene of interest. An additional challenge is representation of some genes that use the same coding DNA sequence but out of frame, such as p16 and p19/ARF. [411,412] Ultimately, the goal by any of these variants is to detect or monitor genome-wide gene expression as an index of total gene activity and, with suitable data mining tools (see below), use that information to better estimate the three cornerstones of oncology: diagnosis, prognosis, and therapy. The general paradigm is equally applicable to any disease state, or biologic function, for that matter.

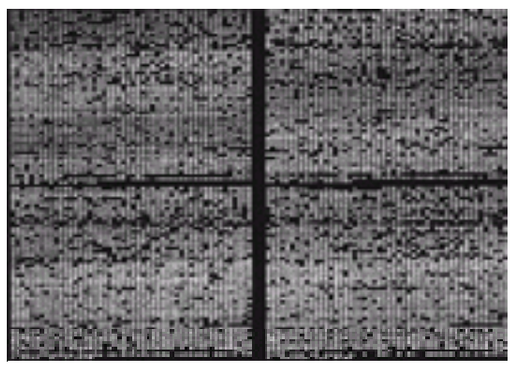

**FIGURE 8-23.** DNA gene expression chip [spotted complementary DNAs (cDNAs)]. An alternative technology, widely used in many research labs, employs full-length cDNAs spotted on glass slides, as seen here. Similar to the technique illustrated in Figure 8-22, tens of thousands of genes can be analyzed in one pass in this fashion. Notably different, however, is the need for the user to define the content of these arrays, as well as the need for competitive hybridization between two samples, one of interest and the other a reference standard. An advantage of the spotted technology is the relative speed whereby final data can be obtained in a matter of hours. A relative disadvantage is the general need for greater amounts of RNA. In reality, both methods are useful and are in widespread use.

Regardless of the specific method used, the information returned is the same: a somewhat quantitative measure of the amount of any given gene being expressed in the sample of interest. When scaled to thousands of genes accessed simultaneously, as current arrays do, a snapshot of the activity of the genome in question is obtained. [413,414] It is increasingly clear that microarray data may be the embodiment of the axiom, “be careful of what you wish for, because you may get it.” Biologists and physicians have yearned for such all-encompassing information about disease states; it is now a reality, but with an enormous caveat: how does one practically use this information? Therein is the conundrum that currently faces everyone who presumes to use this technology. [405]

The great power of this new technology lies in its ability to provide a complex, all-encompassing image of the biologic activity of the cells or tissue at hand. [415] The challenge is to meaningfully analyze the enormous amount of data generated. [416,417,418 and 419] The rapid adoption of this technology by life science researchers has necessarily fostered a parallel effort to develop suitable data mining tools to distill useful information from these arrays. [33,409,414,417,420] Simple scatter analyses with a list of “outlier” genes that are up- or down-regulated in a disease state have limited utility ( Fig. 8-24). In contrast, clusters of genes statistically associated with a given disease, stage, or clinical behavior can be a powerful analytic tool. [32,34,35,419,421,422 and 423] For example, comparisons of three genetic variants of alveolar RMS, PAX3-FKHR, PAX7-FKHR, and translocation negative, have shown a greater similarity of translocation-negative cases to PAX3 cases than to PAX7, which parallels the observed clinical behavior. [349] This observation suggests that these arrays have the inherent potential not only to identify (or “diagnose”) specific diseases but also to identify prognostic groups within a given tumor group. This information, gleaned from analysis of pretreatment biopsy tissue, offers the prospect of predicting clinical course, and therefore optimal therapy, even in the absence of other diagnostic or prognostic markers, before therapy or evolution of the disease.

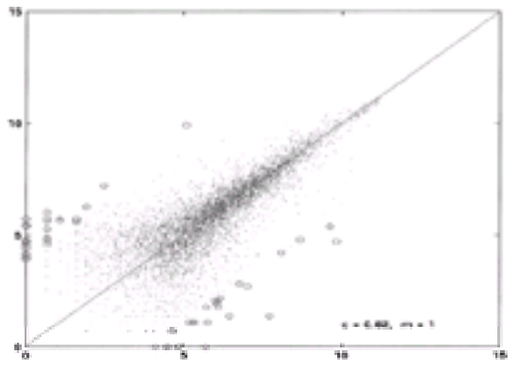

**FIGURE 8-24.** Scatter analysis of expressed genes in rhabdomyosarcoma. Gene expression data can be represented in many forms. A common method is scatter analyses, wherein the quantitative expression value for the same gene for each of two samples is plotted as an x-y coordinate. If all genes were identical in expression between the two, a perfect straight line through the origin with a slope of 1 would result (e.g., a linear regression line). The tendency for two such samples in this illustration is very clear (in fact this represents two physically different portions of the same tumor-analyzing parallel). There is near identity, as indicated by the regression line slope of 1 and a correlation coefficient of 0.82. Note, however, the wide variation in expression of genes expressed at low levels, seen in the lower left portion of the diagram. Distinction between biologic or other noise and true differences in gene expression are difficult to assess in this region. However, the ability to assess effectively all active genes in the human genome simultaneously is a powerful tool that is enjoying increasing acceptance for research and (soon) clinical prognostic purposes.

This is a rapidly evolving field and the next several years will almost certainly demonstrate many clinically useful applications of this technology. Likewise, the current prohibitive cost or technical obstacles will likely be subsumed by facile, inexpensive, and specific diagnostic/prognostic arrays produced from a knowledge of critical genes that dictate clinical behavior in a given disease. If true, these DNA arrays will revolutionize the evaluation of the cancer patient.

### Nucleotide Polymorphisms

Beyond DNA expression arrays lies a vast new arena of genomic analysis, focused on normally occurring genetic polymorphisms, often referred to as *single nucleotide polymorphisms* (SNPs).[407,424,425] These "normal" variants are thought to be associated with variably functional gene products, especially when they occur within coding regions or within 5' promoter/suppressor elements of genes, which in turn may be associated with disease susceptibility, unique drug responsiveness, or clinical evolution of a disease state.[424] The recent sequencing of the entire human genome will result in a vast amount of information regarding normal sequence variation within individuals, which can be represented on so-called SNP chips. These arrays are currently capable of identifying more than 1,500 SNPs, and the number will certainly increase by orders of magnitude shortly as information from the human genome project is used to create vast numbers of SNP arrays. This information, correlated with clinical behavior, will provide yet another picture of clinical behavior in a patient, independent of, but perhaps associated with, their gene expression profiles.[424] Only preliminary studies have been published to date, but the promise of this new technology is clear; when combined with expression and other data, the impact may be staggering.[426,427]

### Proteomics

Finally, the next generation of gene expression profiling will likely be based on protein expression, or proteomics. [428,429 and 430] Although no technology comparable to DNA hybridization currently exists, many competing technologies are being evaluated to accomplish the same result. [431,432,433 and 434] Here, in addition to the basic presence or absence of a gene/protein, one will also be able to assess posttranslational modifications such as glycosylation and phosphorylation, both known to potentially dramatically alter the functionality of a given gene product. These parameters cannot be addressed by a purely nucleic acid method such as the current expression arrays—thus the interest in proteomics.

Whatever the result, a similar picture to gene expression profiles will emerge, but now specifically and quantitatively linked to the amount of effecting protein. Initial studies of tumors have been of some interest, but fundamental insights will require further maturation of the technology, which is still in its early stages. [435,436 and 437] An additional caveat relates to informatics; the vast amount of information from genomics is paralleled with that from proteomics, with comparable challenges, especially when attempts are made to correlate the two.[430,438,439]

## TOPICAL ISSUES IN THE DIAGNOSIS OF CHILDHOOD CANCER

Many tumor-specific issues have been overlooked in the preceding general discussion of childhood tumor diagnosis. A brief consideration of the unique features of specific tumors, particularly where notable breakthroughs or challenges have been described, is warranted. This is intended to give a perspective on the issues of interest as they relate to diagnosis, and in some cases, prognostic relevance. More detailed discussions are found in the chapters dealing with these specific tumors.

### Are Fusion Genes Diagnostic among Childhood Tumors?

The widespread occurrence of chimeric genes associated with specific leukemias and solid tumors of childhood superficially suggests that these tumor-associated aberrant genes are perhaps causative and therefore diagnostic.[253,254,440,441] This assumption rests on two basic premises: (a) the specific gene occurs in a given tumor on a more or less one to one basis, and is therefore tantamount to diagnostic, and (b) the gene is functional and drives phenotypic features that are the basis of historical and current tumor diagnosis. Examples of the first case are well known: *SYT-SSX* in SS; *EWS-WT1* in DSRCT; *EWS-FLI1* or equivalent in EFTs.[442,443,444 and 445] It is, however, important to consider the veracity of this basic assumption as a universal rule, as all diagnoses, and therefore therapy, may be predicated on it. Diagnosis, after all, is the necessary first step in patient management, and the relative precision of molecular diagnostics is therefore of some importance, particularly if information from these molecular diagnostics, as described above, is to supersede that from more conventional methods. In essence, if morphologic diagnostic criteria are in conflict with molecular information, which do you believe?

A consideration of the accumulated literature to date suggests caution in blind faith acceptance of molecular data as primary diagnostic criteria. Two specific problems arise: (a) certain tumor-specific translocations occur only in some percentage of tumors within the group, and (b) in some cases, the same translocation occurs in another tumor, phenotypically distinct from the common type. The first example is epitomized by ARMS, in which the two common fusion genes, *PAX3-FKHR* and *PAX7-FKHR*, are detected in approximately 75% of cases; the remaining 25% of cases show no detectable *PAX-FKHR* fusion (although a novel FKHR analogue may be involved in rare cases) (Sorensen et al., in press). This is echoed in many leukemias, in which the specific chimeric gene may be present in many but not all cases of a defined phenotype (e.g., *TEL-AML1* in 22% acute lymphocytic leukemia).[446]

The second situation occurs when a known fusion gene occurs in an appropriate setting. Examples of this include three different reports of various EWS fusion genes in DSRCT, in one case "resulting" in a mixed DSRCT/EFT tumor phenotype.[447,448 and 449] Note that these examples are not to be confused with those found in certain biphenotypic tumors with a myogenic phenotype and a EFT translocation.[343] These tumors may well be functionally associated with the known common PAX3/7 role in normal and perhaps neoplastic development of both skeletal muscle and primitive neural tissue.[450]

Another example is the appearance of the similar or identical chimeric gene in tumors of different histogenesis. Inflammatory myofibroblastic tumors, which are benign or low-grade malignant neoplastic mesenchymal proliferations with an inflammatory component that can occur in children, demonstrate fusion of tropomyosin 3 or 4 (*TPM3* or *TPM4*) to the *ALK* gene on chromosome 2.[451] *TPM3-ALK* gene fusions have also been described in ALCL.[354, 355] This is reminiscent of *ETV6-NTRK3* fusion transcripts, which in addition to being expressed in CFS and cellular CMN as described, have been reported in a case of acute myelogenous leukemia occurring in a 59-year-old adult patient.[452] Therefore ETV6-NTRK3 and TPM3-ALK, both of which are chimeric PTKs, each appear to be able to transform both mesenchymal and hematopoietic human cell lineages.

What, then, is the relative value of gene translocation analysis in the diagnosis of these often phenotypically undeveloped tumors? The answer is a resounding confirmation of their enormous value in the differential diagnosis of this difficult tumor group.[252,253] The exceptions are so rare as to be reportable and would generally be detected by a simple review of the morphologic appearance. This is a far better accuracy than is normally associated with the conventional diagnosis of these tumors. In fact, in most studies careful review of the "aberrant" cases reveals an incorrect initial diagnosis, not an invalid molecular diagnostic.[252,323] Fusion gene analysis in childhood solid tumors therefore should be viewed as superior to any other diagnostic means to date—although this information should always be used in context with conventional diagnostic criteria. Otherwise, one will draw easily avoidable incorrect conclusions in at least a minority of cases.

### Does Fusion Gene Subtype Analysis Provide Prognostic Information?

It can be said that by virtue of providing a more definitive diagnosis for a given tumor subtype and hence facilitating early institution of appropriate therapy, molecular detection of tumor-specific gene fusions is by definition prognostically relevant. In addition, however, a number of studies suggest that different fusion subtypes within classes of gene fusions might also be independently predictive of outcome.

#### *Ewing Family Tumors*

Virtually all EFTs express some form of *EWS-ETS* gene fusion. Detection of these rearrangements by RT-PCR assays is, as discussed above, very useful as a diagnostic modality for the pathologic workup of EFTs. Further diversity of *EWS-ETS* gene fusions is conferred by different combinations of exons from *EWS* and its partner genes, giving rise to variably sized fusion products.[339] A recent analysis limited to *EWS-FLI1*–positive cases revealed a significantly better prognosis in patients with the more common type 1 gene fusion (*EWS* exon 7 fused to *FLI1* exon 6) in comparison to cases with larger, less common fusion types (Fig. 8-25).[453,454] This difference was only seen among cases with localized disease. In another recent study, no association with outcome was found when *EWS-FLI1*-expressing tumors were compared to *EWS-ERG*-expressing tumors.[455] Further studies will be necessary to determine whether the prognostic significance of smaller versus larger

*EWS-ETS* fusion types also extends to the less common variant fusions such as *EWS-ERG*, in which the number of cataloged cases with clinical follow-up for each fusion subtype is small and difficult to interpret with regard to meaningful outcome analysis.

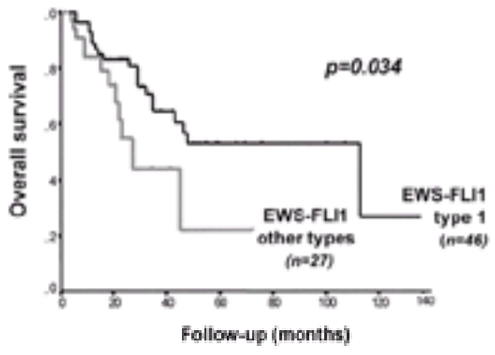


**FIGURE 8-25.** Ewing's survival by fusion gene type (type 1 versus others). Differences in clinical behavior observed in Ewing's sarcoma patients whose tumors express an EWS-FLI1 type 1 translocation as opposed to all other types of EWS-FLI1 translocations have been reported by two authors. [456,457] Data showing a more favorable survival for patients with an EWS-FLI1 translocation with a *p* value of .03 in this study of 73 patients are reproduced here. [457] (Reproduced with the permission of Mark Ladanyi. de Alava E, et al. EWS-FLI1 fusion transcript structure is an independent determinant of prognosis in Ewing's sarcoma [Published erratum appears in J Clin Oncol 1998;16:2895] [see comments]. J Clin Oncol 1998;16:1248–1255.)

### *Synoviosarcoma*

In a recent study of SS, *SYT-SSX1* and *SYT-SSX2* fusion transcripts were detected in 64% and 36%, respectively, of a series of 45 SS cases. [456] There was a significant relationship between histological subtype: all 12 biphasic SS had a *SYT-SSX1* fusion transcript, and all 16 tumors that were positive for *SYT-SSX2* were monophasic. Furthermore, patients with *SYT-SSX2* had significantly better metastasis-free survival than did patients with *SYT-SSX1*; histological subtype alone was not prognostically important.[456] Similar findings have been reported by others.[457] Therefore it appears that the type of *SYT-SSX* fusion transcript correlates with both the histological subtype and the clinical behavior of SS. A new variant *SYT-SSX* gene fusion, *SYT-SSX4*, has recently been reported[458]; it will be interesting to determine its incidence in SS and how it relates to outcome in this tumor class.

### *Alveolar Rhabdomyosarcoma*

ARMS remains a devastating disease clinically and a formidable challenge for the pathologist attempting to make the diagnosis. The poor outcome for ARMS has led to an intense search for prognostic indicators useful for therapeutic stratification. As described above, t(2;13) and t(1;13) chromosomal translocations resulting in the expression of two related fusion genes, *PAX3-FKHR* and *PAX7-FKHR*, respectively, are characteristic of ARMS. Detection of *PAX3-FKHR* and *PAX7-FKHR* fusion transcripts in tumor specimens by RT-PCR assays is widely accepted as a useful tool in the diagnostic workup of ARMS. However, up to now, the incidence of these abnormalities and their specificity for ARMS have not been examined in a large cohort of centrally reviewed RMS cases. In a very recent report, RT-PCR analysis of *PAX3-FKHR* and *PAX7-FKHR* gene fusions in 171 RMS cases from Intergroup Rhabdomyosarcoma Study-IV (IRS-IV) detected fusion transcripts only in ARMS (Sorensen et al., in press). No ERMS cases or tumors diagnosed as undifferentiated sarcoma were fusion positive, confirming the specificity of these alterations for ARMS. Interestingly, 23% of the 78 ARMS cases tested were fusion negative, suggesting that other genetic abnormalities such as *PAX-FKHR* variant gene fusions may characterize at least a subset of ARMS. Consistent with an earlier preliminary report, [349] this study also found a correlation between fusion status and clinical outcome. Among ARMS cases presenting with metastatic disease there was a striking association between *PAX3-FKHR* expression and poor outcome (Sorensen et al; in process citation). The estimated 4-year overall survival rate was more than 70% for *PAX7-FKHR* versus less than 10% for *PAX3-FKHR* in cases presenting with metastatic disease. Multivariate analysis confirmed this finding: there was a significantly increased risk of relapse and death in patients with metastatic disease if their tumors expressed *PAX3-FKHR*. This was coupled to a very intriguing difference in the pattern of metastatic disease between *PAX3-FKHR*– and *PAX7-FKHR*–positive cases: more than 50% of metastatic *PAX3-FKHR*–positive ARMS patients had bone marrow metastases, whereas none of the metastatic *PAX7-FKHR*–positive cases demonstrated bone marrow involvement. Therefore expression of *PAX3-FKHR* and *PAX7-FKHR* gene fusions identify high-risk and low-risk subgroups, respectively, among ARMS patients presenting with metastatic disease.

## What Is Poorly Differentiated Rhabdomyosarcoma?

Two major issues surround the diagnosis of RMS: is the tumor in question a RMS, and if so, what type? These questions, as it turns out, are not trivial, because the answer is frequently wrong in both cases, with unfortunate consequences for the patient. Historical experience has shown that these tumors are best treated on the protocols developed over the past quarter century by the former Intergroup Rhabdomyosarcoma Study Group. Patients with RMS treated otherwise often fare less well.

### *Rhabdomyogenesis*

Morphologic methods of detecting skeletal muscle differentiation in a sarcoma fail with alarming regularity, as became clear when better methods such as immunohistochemistry for muscle proteins became available. For several years now, the standard repertoire has included two main antibodies (as discussed in more detail previously): desmin and muscle actin. However, neither is unique to skeletal muscle; desmin occurs widely in nonrhabdomyogenic cells (including myofibroblasts), and muscle actin routinely reacts with smooth muscle. Thus, myogenesis may be assumed, but *rhabdo*myogenesis cannot. What, then, to do? Developmental studies of transcription factors have documented the critical and pivotal role these genes play in normal skeletal muscle development; the same is true of rhabdomyogenic tumors. MyoD and Myogenin in particular are useful markers of incipient rhabdomyogenesis; MYF5 is as well but is less widely used as a marker. A typical diagnostic method has been RT-PCR to detect these rare mRNA transcripts. PCR can be disastrously misleading, however; even the most minute contamination from normal skeletal muscle (a likely event in a sarcoma, it should be noted) will yield a false-positive result in nonmyogenic sarcomas. Antibodies have been developed against MyoD and Myogenin that are able to detect even these scant transcription factor proteins in the nucleus, either on frozen tissue sections or on imprints. This is illustrated in Figure 8-13A. More recently, antigen retrieval methodology has extended this to ordinary formalin-fixed, paraffin-embedded tissue sections as well.[24] A positive result here it should be noted, especially if localized to tumor cell nuclei, is unequivocal evidence of rhabdomyogenesis, unlike the ambiguous results given by desmin and muscle actin antibodies. This, then is the preferred diagnostic marker for this tumor.

### *Classification*

Having established that a questionable tumor is RMS, the next challenge is often how to subclassify the tumor. Such cases are generally *not* typical examples or they would not have been subjected to such scrutiny in the first place. Although some are spindle cell tumors, the more common variety is a round cell tumor, the same round cell tumor problem discussed earlier. In this case, the issue is which category of RMS, referencing the recently published International Classification of Rhabdomyosarcoma summarized in Table 8-5. The single most important change from historical systems (for the purposes of this discussion) is the recognition that alveolar RMS need not display an alveolar pattern. The diagnosis of this type of RMS is more cytologic and molecular genetic (although approximately 25% of cases will be translocation negative, necessitating reliance on morphology for their diagnosis when no alveolar pattern is present). Strikingly, the major reviews of IRSG study material that developed this schema documented both a failure to recognize alveolar cytology in nearly one-third of alveolar RMS cases and a marked increase in the different prognoses of these two tumors when this mistake was corrected. After review, the number of alveolar RMS cases increased nearly 50%, to one-half as common as the more common ERMS, from one-third, as was common by historical criteria. At the same time, survival differences became statistically significant, with alveolar RMS faring worse, as long suspected. A typical example of the cases incorrectly identified as ERMS, but now recognized as a variant of alveolar RMS termed *solid alveolar RMS*, is shown in Figure 8-26.

| Diagnosis | Histology | Prognosis |
|---|---|---|
| Botryoidal embryonal | Favorable | Superior |
| Spindle cell embryonal | Favorable | Superior |
| Embryonal, not otherwise specified | Favorable | Intermediate |
| Alveolar | Unfavorable | Poor |
| Solid alveolar | Unfavorable | Poor |
| Anaplastic/pleomorphic embryonic | Unfavorable | Poor |
| Undifferentiated sarcoma | Unfavorable | Poor |

**TABLE 8-5. INTERNATIONAL CLASSIFICATION OF RHABDOMYOSARCOMA**

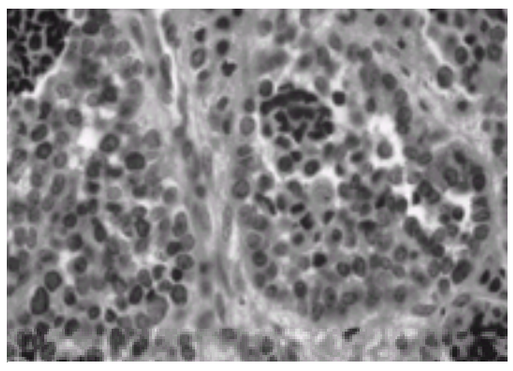

**FIGURE 8-26.** Solid alveolar rhabdomyosarcoma. This tumor in the Intergroup Rhabdomyosarcoma Studies was routinely misdiagnosed as a form of embryonal rhabdomyosarcoma for nearly 25 years. As a result of the new international classification scheme, 10% to 20% of all cases have now been correctly reclassified as alveolar rhabdomyosarcoma. As a group they have histology similar to that seen here, composed of round, poorly adherent cells that may begin to form a nascent alveolar pattern (*right side*). The true alveolar character of these lesions has been documented: they show a similar incidence (i.e., approximately 75%) of a PAX3- or PAX7-FKHR translocation, as observed in conventional forms of the disease.

***Lineage Fidelity and Fusion Gene Status***

We have previously identified *EWS-ETS* gene fusions identical to those found in EFTs in a series of myogenic solid tumors occurring in the soft tissues of children and young adults.[343] These tumors had an SRCT morphology with histological features suggestive of ARMS. Moreover, they expressed myogenic cell intermediate filaments. In one case, the initial presentation was indistinguishable from an EFT, but after therapy both Ewing's-like and frankly rhabdomyoblastic elements were detected (Fig. 8-27A, Fig. 8-27B and Fig. 8-27C). This case tested positive for EWS-FLI1 fusion transcripts ( Fig. 8-28), documenting the EFT lineage; the frank myogenesis documented the rhabdomyogenic lineage.

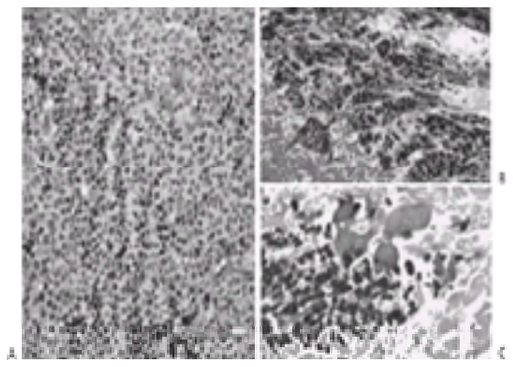

**FIGURE 8-27.** Biphenotypic sarcoma (Ewing's plus rhabdomyosarcoma). The three panels in this illustration show **(A)** the pre-therapy appearance of the tumor, which was composed entirely of round, undifferentiated cells that appeared to be those of Ewing's sarcoma. After treatment and subsequent excision, the tumor now showed evidence of two cell types: **(B)** continued to resembled Ewing's sarcoma, whereas some portion of the tumor **(C)** now showed unequivocal evidence of terminal rhabdomyoblastic differentiation. Thus the original tumor was too undifferentiated to identify as a mixed Ewing's and rhabdomyosarcoma tumor, but the treatment-induced differentiation revealed the unequivocal dual phenotypic character of the tumor.

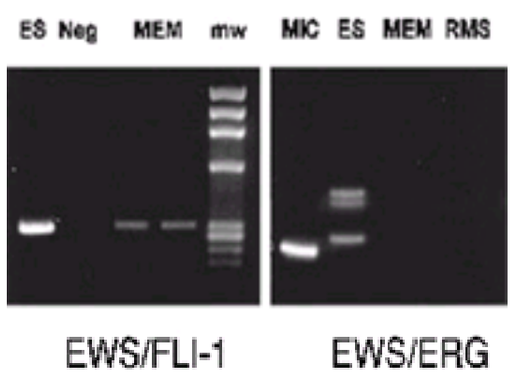


**FIGURE 8-28.** Polymerase chain reaction (PCR) confirmation of the Ewing's translocation. To confirm the distinction between simply primitive or undifferentiated rhabdomyosarcoma versus a true biphenotypic tumor, PCR analysis of the tumor along with control samples, such as rhabdomyosarcoma, is illustrated here. A positive band for *EWS-FLI1* is readily detected, confirming the Ewing's family character of the tumor, but here unquestionably a component of a rhabdomyosarcomatous tumor, as documented in Figure 8-27. This has been termed *biphenotypic sarcoma* by the authors, and is considered a primitive form of the conventional malignant ectomesenchymoma (MEM). ES, Ewing's sarcoma; mw, molecular weight; Neg, negative; RMS, rhabdomyosarcoma.

We have hypothesized that these biphenotypic tumors represent examples of malignant ectomesenchymomas (MEMs), defined as soft tissue tumors having neural sarcomatous elements in addition to a malignant mesenchymal (rhabdomyoblastic) component[459,460 and 461] and that these tumors are members of the pPNET family, now EFTs.[343] This finding is potentially of importance diagnostically as at least some *PAX3-FKHR* or *PAX7-FKHR* fusion–negative ARMS cases may represent MEMs. In a recent analysis of approximately 200 cases originally submitted to IRS-IV for molecular studies and possible inclusion on IRS-IV therapeutic protocols, we did identify one case out of approximately 50 cases with ARMS morphology that was *PAX3-FKHR* or *PAX7-FKHR* fusion–negative but which expressed an *EWS-FLI1* gene fusion (P.H.B. Sorensen, F.G. Barr, and T. Triche, *unpublished results*). Therefore, although rare, at least some ARMS cases may actually represent MEMs with

EWS-ETS gene fusions. Alternatively, some ARMS cases lacking ARMS-associated gene fusions may represent cases of IDSRCT in which either neural or epithelial markers are difficult to detect. It is therefore recommended that SRCTs with ARMS morphology but that lack *PAX3-FKHR* or *PAX7-FKHR* gene fusions should be assayed for *EWS-ETS* or *EWS-WT1* gene fusions as part of the diagnostic workup.

**Cellular Fibroblastic Lesions of Early Childhood**

A considerable source of controversy for both pathologists and clinicians is the occurrence of cellular, mitotically active spindle cell tumors in very young children, which have the morphologic appearance of ATFS.[462] Fibrosarcoma is known to occur in children, but there appears to be a bimodal age distribution to this entity. Those occurring before age 5 years (with most occurring in children younger than 1 year) are known as *CFS*.[362] A second peak occurs in patients aged 10 to 15 years, and these are referred to as *ATFS*. ATFS is histologically identical to CFS, and the lower end of the pediatric age range in ATFS is not well defined, making the distinction from CFS potentially problematic in young children. This is an important distinction; whereas CFS generally has an excellent prognosis and is often treated with surgery alone, ATFS is an aggressive lesion with a poor prognosis similar to that of adult fibrosarcoma. [462] Both CFS and ATFS may be confused with fibromatosis, a less cellular proliferation of benign-appearing fibroblasts with a more collagenous matrix ( Fig. 8-29).[362] This lesion most commonly occurs in children aged 2 years or younger and is therefore often referred to as *infantile fibromatosis* (IFB). When IFB cells show contractile elements ultrastructurally or stain for muscle actin suggesting myofibroblast derivation, the term *myofibromatosis* is used.[362,463] This lesion often demonstrates multifocal sites of involvement. Aggressive fibromatosis is a clinical term for an otherwise histologically indistinguishable form of IFB that shows increased local invasion or recurrence rates. [462] Therefore CFS may be confused with a number of both benign and malignant entities. Unfortunately, standard pathologic examination often does not readily distinguish these conditions from each other.

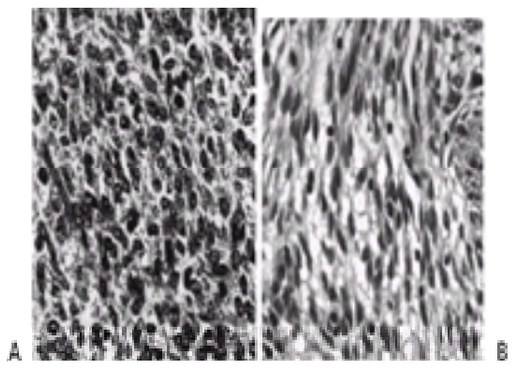


**FIGURE 8-29.** Light microscopic appearance of fibrosarcoma and fibromatosis. **A:** A typical fibrosarcoma, with characteristic darkly basophilic nuclei and disorderly growth pattern contrasts strongly with the spindle cells of fibromatosis. **B:** The striking dichotomy seen here is not present in all cases, and overlapping appearances sometimes render the two forms indistinguishable. Hematoxylin and eosin, ×400.

As described, a novel t(12;15)(p13;q25) chromosomal translocation has recently been described in CFS that gives rise to an oncogenic *ETV6-NTRK3* gene fusion.[364] RT-PCR assays can detect *ETV6-NTRK3* fusion transcripts in CFS frozen[364] or paraffin-embedded tumor.[366,464] In a recent series of 62 predominantly pediatric soft tissue spindle cell tumors, including 11 CFS cases, 13 malignant spindle cell tumors (including ATFS) and 38 benign spindle cell tumors (including IFB and myofibromatosis), Bourgeois et al.[464] found that *ETV6-NTRK3* was expressed only in the CFS cases. In this study, the authors also examined whether immunohistochemistry with an antibody to the NTRK3 PTK domain could detect ETV6-NTRK3 proteins in paraffin-embedded tissue blocks; however, this technique did not reliably detect chimeric proteins in tumor specimens. Therefore RT-PCR detection of the *ETV6-NTRK3* gene fusion in pediatric spindle cell tumors appears to be the modality of choice for identification of *ETV6-NTRK3* gene fusions in the diagnostic workup of CFS. It is hoped that further genetic characterization of other pediatric spindle cell tumors will provide better markers for these diseases as well.

**Nonrhabdomyogenic Spindle Cell Sarcomas of Older Children**

A common problem in older children is the distinction of fibrosarcoma, monophasic SS, and malignant nerve sheath tumors from one another. These tumors, all nonmyogenic spindle cell sarcomas, are conceptually quite distinct. Further, the biologic or clinical behavior is also allegedly quite different; most MPNSTs are considered to be of low-grade malignancy, ATFS is a full-blown malignancy, and SS is generally a high-grade malignancy. In reality, the distinction between these entities has increasingly been questioned in view of recent data linking at least some proportion of these three tumors.

ATFS, unlike the congenital form discussed previously, generally occurs in adolescents and young adults, is not a variant of the common malignant fibrous histiocytoma of adults, and requires aggressive surgery and potentially adjunctive therapy (although well-accepted, multimodality therapy protocols are not widely used).[465,466 and 467] No diagnostic gene fusion or other marker has been identified, unlike the histologically similar but pathogenetically unrelated and only recently recognized as malignant (albeit low-grade) soft tissue tumor, inflammatory myofibroblastic tumor. [451,468] For some years, then, fibrosarcoma has been a seemingly clear cut entity, a bona fide member of the nonRMS soft tissue sarcomas.[467]

SS has for years been recognized in its classic biphasic form, as well as the less typical monophasic form, which can be difficult to distinguish from fibrosarcoma ( Fig. 8-30).[469,470] Remarkably, a consistent chromosomal translocation unique to this tumor was identified in 1986 by Turc-Carel et al. [357] and subsequently confirmed by a number of others.[471,472,473,474 and 475] Within a short time, the breakpoint was cloned and shown to involve two unique genes, *SYT* on chromosome 18 and a novel gene, *SSX*, on chromosome X.[476,477] Shortly thereafter, two gene transcripts were identified on chromosome X, identified as *SSX1* and *SSX2* (Fig. 8-19).[358,359,478,479] This number has now been expanded to *SSX4*, but only if *SYT* in involved from chromosome 18.[458] Remarkably, this fusion gene has only been found in SS, and in all SS studied to date in which adequate material has been available. [358,359,444,476,477,478,479 and 480] Thus, the presence of an *SYT-SSX* gene fusion is thought to be 100% predictive of a diagnosis of SS.

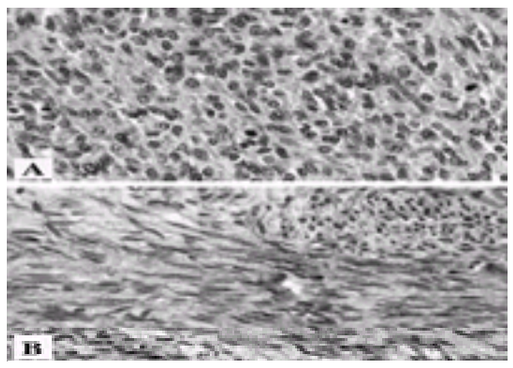


**FIGURE 8-30.** Fibrosarcoma versus monophasic synoviosarcoma. Spindle cell tumors, even when clearly malignant, can be difficult to distinguish. Here a typical fibrosarcoma **(A)** and a typical monophasic synoviosarcoma **(B)** are illustrated. At first glance, it is frequently difficult to distinguish the two; before the advent of molecular methods (Fig. 8-19), monophasic synoviosarcomas were routinely identified as fibrosarcoma due to their typically fibroblastic appearance as seen here. When compared to a conventional fibrosarcoma **(A)**, however, striking differences are evident, notably the wavy nuclei and fibrillar stroma of synoviosarcoma.

The third entity to be considered here is MPNST. Historically, this was often termed *neurofibrosarcoma* or *malignant schwannoma*, particularly when it occurred in

adults.[481,482,483,484,485 and 486] In children, unlike adults, there is no association with von Recklinghausen's disease or abnormalities of NF1. Numerous features, from EM evidence of perineural cell differentiation with basal lamina formation to S100 immunoreactivity by immunohistochemistry, appeared to distinguish this tumor from fibrosarcoma or SS (Fig. 8-31A, Fig. 8-31B).

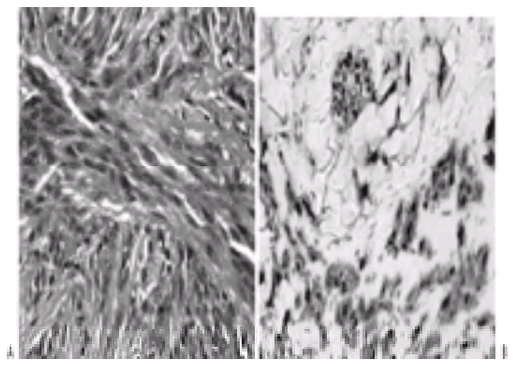

**FIGURE 8-31.** Malignant peripheral nerve sheath tumor. It is increasingly recognized that malignant peripheral nerve sheath tumor **(A)** can be difficult to distinguish from fibrosarcoma and synoviosarcoma. Immunohistochemistry for S100 protein **(B)** will generally identify malignant nerve sheath tumor, is only rarely reactive with synoviosarcoma, and should be nonreactive with fibrosarcoma. Increasingly, however, these analyses are being augmented with *SYT-SSX* analysis for synoviosarcoma. However, exceptions to this dichotomy (e.g., *SYT-SSX*–positive synoviosarcoma with S100-positive cells) have been reported.

The diagnostic overlap central to this discussion first appears with the description of tumors that appear to be nerve sheath tumors but with divergent differentiation, forming skeletal muscle (so-called Triton tumors) or glandular elements (or glandular differentiation in MPNST).[487,488 and 489] This then raises the question of how to distinguish a malignant nerve sheath tumor with glandular differentiation from a biphasic SS. The presence of the diagnostic *SYT-SSX* translocation can potentially distinguish the two. However, as more of these tumors are analyzed, particularly those spindle cell tumors expressing neural markers but lacking glandular differentiation, examples with the same SS-specific translocation have been encountered.[194,490,491]

Either there is a link between these three tumors or there is inappropriate expression of neural markers by some SS and fibrosarcomas. In either case, molecular genetic analysis has provoked a reconsideration of the classification of these seemingly unrelated tumors. This is particularly unexpected given the general association of SS with a highly unique and possibly pathogenic chromosome translocation, in contrast to the common association of malignant nerve sheath tumor with NF1 mutations or loss. Given the seemingly different behavior between these tumors, it will be important to use phenotypic and molecular genetic methods to resolve this ambiguity. In the meantime, an *SYT-SSX* translocation analysis is needed in every suspected case of SS, and ATFS and MPNST as well.

**Is the Rhabdoid Tumor a Tumor or a Phenotype?**

The rhabdoid tumor was first described as an unusual variant of Wilms' tumor in early National Wilms' Tumor Study Group studies.[492] Notable features were an extraordinarily prominent nucleolus, and to a lesser extend, brightly eosinophilic cytoplasm resembling that of RMS cells (hence the name) ( Fig. 8-32A). These features are easily confirmed by EM as well (Fig. 8-32B). At first, these tumors were thought to be limited to the kidney, but within a short time they were identified in almost every anatomic location.[493,494,495,496,497,498,499 and 500] Worse, they often occurred superimposed on bona fide tumors of otherwise known lineage, or at least diagnosis, such as SS, leading some to suggest such tumors were not a tumor per se, but rather a particularly poor prognosis form of any sarcoma (as almost all such patients died in early reports).[498,501,502]

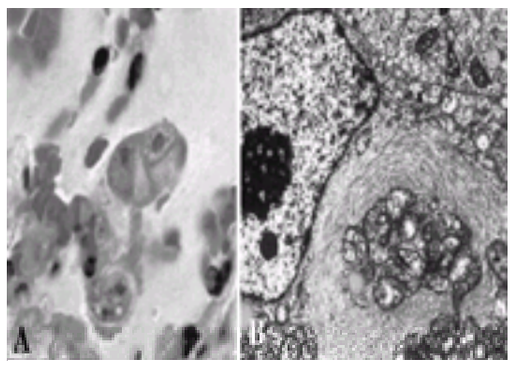


**FIGURE 8-32.** Rhabdoid tumor—cytology and electron microscopy (EM). This “tumor” has been historically identified based on unique histiologic and cytologic characteristics. Typically the cells show a prominent nucleolus and a cleared area of adjacent cytoplasm, often with eosinophilic inclusions **(A)**. By EM **(B)** the cytoplasmic inclusion is found to be a whorl of intermediate filaments. The prominent nucleoli are also evident by EM. Recent description of a common *SNF5* (*INI1*) mutation in this tumor suggests that it is a recurring genomic alteration in a tumor, but the frequent occurrence of the phenotype in other well-characterized tumors suggests that the phenotype can exist independent of the tumor type (e.g., rhabdoid phenotype in synoviosarcoma with a characteristic SYT-SSX chimeric gene).

With time, a unique chromosomal translocation involving the short arm of 11 and the long arm of 22 was described [and thus distinct from the Ewing's tumor t(11;22), which involves the long arm of each chromosome], although the chromosome 22 breakpoint in the rhabdoid tumor was exceedingly close to the EWS breakpoint of Ewing's tumor.[503,504] Numerous attempts to identify a chimeric gene analogous to that described in RMS or Ewing's, for example, failed.[505,506,507,508,509,510 and 511] Recently, however, a unique genetic defect has been described, specifically the loss or mutation of snf5 (or INI1), a member of a family of genes important in chromatin remodeling.[512,513] The common and necessary finding of a single prominent nucleolus would thus seem to be linked in some fashion to defective function of this gene complex in these tumors, although a specific functional relationship between this genetic abnormality and the ubiquitous prominent nucleolus is currently unclear. Why most tumors also accumulate unique cytoplasmic whorls of intermediate filaments is even less clear. Nonetheless, the alteration at the moment appears restricted to rhabdoid tumors, but this, too, may prove illusory, as have most “tumor-specific” gene abnormalities. (B. Weissman, *personal communication*, 2000) Despite this, SNF5 mutations appear to be found in most if not all rhabdoid tumors, suggesting an etiologic role for this defect. Widespread diagnostic testing awaits further clarification of the common defect in all such tumors; no specific translocation or mutation akin to those in other sarcomas has yet been described.

## A FINAL WORD

It should be apparent from the foregoing that diagnosis of tumors and tumorlike conditions, or even the exclusion of the same, is uniquely difficult in children, with disproportionate consequences for an incorrect diagnosis. Presumably for this reason, many of the most specialized diagnostic modalities introduced into medicine have been first applied to childhood tumors. Molecular genetic analysis of hematopoietic malignancies (notably leukemia and lymphoma) is not routine as is gene amplification and quantitative gene expression analysis in NB. PCR is now ordinary, used in almost every case of RMS, EFT, SS, DSRCT, melanoma of soft parts, and others suspected of being any of the above. FISH is also routine for translocations in all of the above.

This is hardly the case in adult tumor diagnosis. Why, and why does it matter? In part, the high frequency of mesenchymal tumors (leukemia, lymphoma, Wilms' tumor, and sarcomas in particular) with their extraordinary frequency of reproducible genetic abnormalities (translocations, deletions, amplifications, and imprinting) clearly contribute to the utility of molecular genetic diagnostics among these tumors. But that only speaks to utility. The real reason is that the results of these diagnostic procedures make a difference in patient management and outcome. Fortunately, many of these diagnostics are closely linked to specific prognoses. *NMYC*

amplified NBs are associated with a poor prognosis, as are *PAX3-FKHR* alveolar RMSs and *SYT-SSX1* SSs.

What comes next will be perhaps the most interesting chapter in this continuing story. Identifying not just single- (or two-) gene abnormalities but wholesale genomic and expression features, as promised by DNA microarrays and similar emerging technologies, offers the real prospect of, for the first time, identifying diagnostic, prognostic, etiologic, and therapeutic target features in a given tumor all at once. This will indeed usher in a new understanding of childhood cancer and likely cancer (and disease) in general.

The rate at which new understanding has come to oncology in general and pediatric oncology in particular within the past decade or so is unprecedented. It will only increase disproportionately with the recent successful sequencing of the human genome and the vast amount of knowledge that will flow from there. With that knowledge comes the responsibility to apply it in a clinically meaningful, useful, and responsible manner. The preceding commentary offers at least some direction for doing so in the diagnostic evaluation of the child with cancer.

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# 9

# IMAGING STUDIES IN THE DIAGNOSIS AND MANAGEMENT OF PEDIATRIC MALIGNANCIES

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## INTRODUCTION

The past three decades have seen a remarkable improvement in the survival rate of children with malignant disorders and witnessed a technologic explosion in the field of diagnostic imaging that has made accurate assessment of tumor size and spread more precise than ever before possible. These advances in imaging technology, however, entail increased knowledge and sophistication on the part of the imaging specialist, greater cost of technologically advanced equipment, and increased difficulty in choosing the appropriate imaging modality for the quickest, most accurate, and least expensive patient evaluation at the lowest possible radiation dose. Since the previous editions of this book were published, improved technologic sophistication rather than wholly new technology has characterized the field of diagnostic imaging. This chapter discusses the innovations of the past several years and explores in greater detail the contributions of increasingly sophisticated computerized imaging procedures. The relative merits of the imaging procedures currently available are considered, and recommendations are made concerning the evaluation of general problems in pediatric oncology. The imaging characteristics and the imaging evaluation of patients with specific tumors are dealt with in the appropriate specific chapters of this text.

## RELATIVE MERITS OF IMAGING PROCEDURES

### Plain Film Radiography

At a time of remarkable advances in sophisticated imaging modalities, the plain film radiograph continues to play a significant role in the diagnosis, evaluation, and follow-up of children with malignant disease ( Table 9-1). Although less sensitive than computed tomography (CT), plain film chest radiography is the examination of choice for short-term interval evaluation of both primary intrathoracic tumors and of pulmonary metastases ( Fig. 9-1). The examination is fast, technically easy to perform, inexpensive, requires no anesthesia or sedation, and delivers minimal radiation to the thyroid, breast tissue, bone marrow, and gonads. The chest film also continues to be the initial procedure of choice for evaluating complications of therapy such as drug reactions and pulmonary infections in the immunocompromised host.

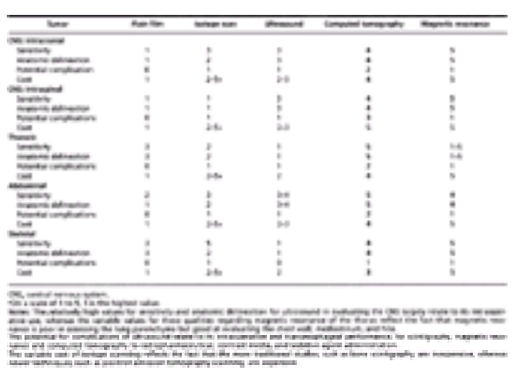

**TABLE 9-1. COMPARATIVE VALUE OF IMAGING PROCEDURES ON A SCALE OF 1 TO 5[a]**

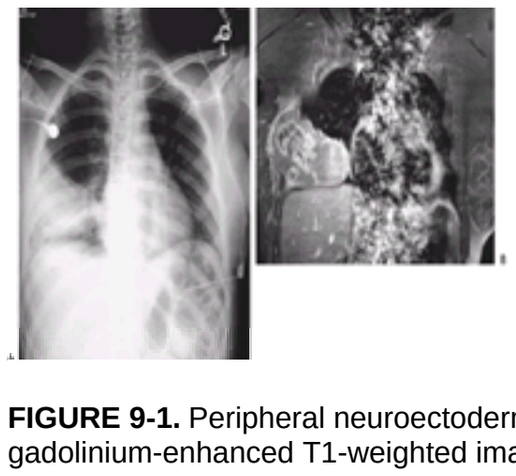

**FIGURE 9-1.** Peripheral neuroectodermal tumor. **A:** Posteroanterior chest radiograph demonstrates a large mass at the base of the right hemithorax. **B:** Coronal gadolinium-enhanced T1-weighted image confirms the presence of the lesion and shows areas of diminished signal within it consistent with intratumoral necrosis.

Plain films of the abdomen have to a great extent been superseded by more technologically advanced procedures for evaluation of abdominal masses and other problems found in pediatric oncologic patients. Nevertheless, sufficient information can often be gleaned from abdominal plain films to warrant their performance before more sophisticated imaging procedures are undertaken. Specifically, although not as sensitive as CT for finding calcification within tumors, the plain film of the abdomen does depict most calcifications and many intraabdominal masses. Finally, and perhaps most significantly, conventional radiography of the abdomen

constitutes the best simple means to evaluate for bowel obstruction and pneumoperitoneum.

Excretory urography is seldom performed to evaluate patients with oncologic disease. Instead, various combinations of nuclear scintigraphy, ultrasound, CT, and magnetic resonance (MR) are used to evaluate retroperitoneal disease, both intrinsic and extrinsic to the genitourinary system.

Barium studies are a simple and sensitive means of evaluating gastrointestinal (GI) tract lesions ( Fig. 9-2). However, with the exception of lymphoma, primary neoplastic involvement of the GI tract is rare in children and, in the case of lymphoma, CT offers the advantage over barium studies of not only demonstrating the bowel but of also depicting the mesentery, the mesenteric lymph nodes, and the solid abdominal viscera ( Fig. 9-3). Mucosal lesions of the GI tract are better seen with traditional barium studies than by any of the more advanced imaging studies available. Esophagitis, duodenal ulcer, drug-induced colitis, and radiation enterocolitis are, for example, best identified by barium studies. Contrast enemas must, however, always be used with caution and are generally contraindicated in neutropenic patients because of the bacteremia that invariably occurs during the procedure ( Fig. 9-4). Contrast studies performed after oral ingestion of contrast agents are generally safe even in immunocompromised children.

**FIGURE 9-2.** Burkitt's lymphoma manifesting as ileocolic intussusception in a 4-year-old boy. Barium enema demonstrates the typical coiled-spring appearance of intussusception.

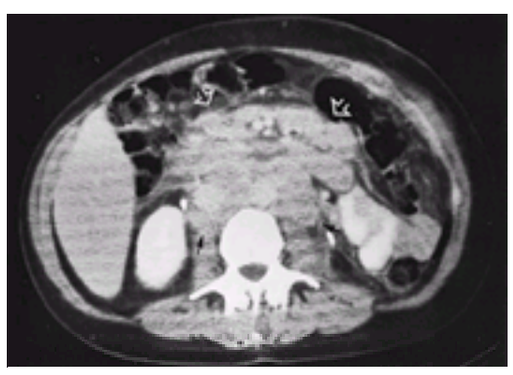

**FIGURE 9-3.** Non-Hodgkin's lymphoma in a 17-year-old girl. Computed tomography demonstrates mesenteric lymphadenopathy (*open arrows*) and retroperitoneal lymphadenopathy (*closed arrows*).

**FIGURE 9-4.** Radiation colitis in 6-year-old boy presenting 2 years after therapy for pelvic rhabdomyosarcoma. Barium enema shows rigidity, narrowing, and mucosal effacement of the sigmoid colon.

Barium is an inexpensive, convenient, and safe contrast medium for the evaluation of the GI tract. Its use is contraindicated, however, when perforation of the intestine or bowel obstruction is suspected. In such circumstances, water-soluble contrast material should be used. Historically, derivatives of meglumine and diatrizoate salts have been used, but these hyperosmolar agents have been largely abandoned due to their propensity to cause dangerous fluid shifts when administered in large quantities, pneumonitis when aspirated, and peritonitis when spilled from the GI tract. Newer, nonionic low-osmolar agents, though expensive, are safer, provide better detail of the GI tract mucosa, and remain undiluted within the GI tract for longer periods.

Skeletal radiographs are reasonably sensitive in detecting bony abnormalities, although most neoplastic and infectious processes are apparent on radionuclide bone scans and by MR imaging before they are evident on plain films. Nevertheless, by the time most patients with primary bone tumors are symptomatic, the plain film image is abnormal and should be used as the first screening modality in a patient with bone pain. MR has proved to be better than plain films, radionuclide studies, or CT in determining the osseous and extraosseous extent of bone neoplasms (Fig. 9-5) and is currently the mainstay for the evaluation of primary bone tumors. MR can be used to assess for metastatic disease but total-body MR imaging is cumbersome and, with the exception of Langerhans' cell histiocytosis [1] and neuroblastoma, in which radiographic skeletal surveys can serve an important complementary role, nuclear scintigraphy is generally the preferred modality for the detection of bony metastases. Of great importance, however, is the fact that, although MR and radionuclide scanning are highly sensitive, these modalities, and especially bone scintigraphy, are considerably less specific than plain film imaging, and thus abnormalities detected by MR or by scintigraphy should be correlated with conventional skeletal radiographs.

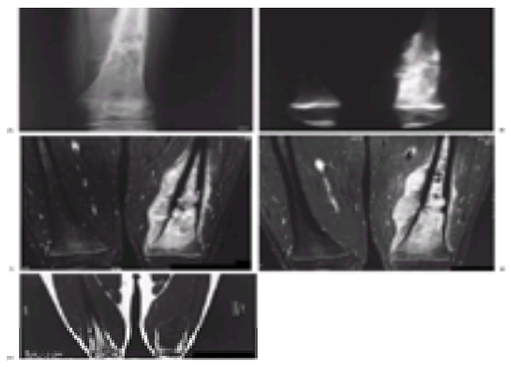

**FIGURE 9-5.** Imaging evaluation of primary bone tumors. **A:** Anteroposterior radiograph of the left femur shows a mixed sclerotic and lytic process in the left femoral shaft with “sunburst”-type periosteal reaction medially, a classic appearance for new bone production in this osteosarcoma. **B:** Anteroposterior 99-m technetium methylene diphosphonate bone scintigram shows increased uptake at the site of the tumor. **C:** Coronal short t inversion recovery magnetic resonance (MR) image shows increased signal at both the intramedullary and extraosseous portions of the tumor. **D:** Coronal T1-weighted MR image shows the signal from the intramedullary tumor to be less intense than that of the normal fatty marrow within the distal portion of the right femur. The extraosseous soft tissue tumor has T1-weighted signal similar to that of normal muscle. **E:** Coronal fat-saturated T1-weighted MR image shows increased enhancement of both the intra- and extraosseous portions of the tumor.

Skull radiography, in the era of CT and MR, is rarely useful. [2] A skull film should always be included as part of a radiographic metastatic survey to detect calvarial metastases, but virtually any other abnormality, including lesions of the sella turcica, sphenoid wings, and the skull base, is better seen on CT or MR. [3,4] Even as a screening mechanism, plain skull radiography adds nothing but cost to the evaluation of the patient and can always be obtained after the CT or MR if there is reason to think it might add some information.

Spinal radiographs are valuable as a screening examination for metastatic disease and for compression fractures secondary to disease-induced or drug-induced osteoporosis. Radioisotope bone scanning, however, is more sensitive for the early detection of metastatic disease or occult bone tumor, and CT or MR gives better definition of bony spinal lesions and of associated abnormalities of the intraspinal contents.

Conventional fluoroscopic myelography was initially largely supplanted by spinal CT performed after intrathecal contrast injection and was subsequently nearly completely replaced by MR imaging, the current study of choice for the evaluation of lesions of the spinal cord and its coverings. [5] MR studies are more diagnostic, are obtained without the intrathecal injection of contrast material, and are generally better tolerated by patients. Of particular importance for children is the fact that MR is extremely sensitive to the encroachment of paraspinous neurogenic tumors through the neural foramina into the spinal canal ( Fig. 9-6).[6] As both MR[7] and CT images can be significantly degraded by the presence of metallic hardware on occasion, conventional myelography can be helpful in evaluating patients with such hardware.

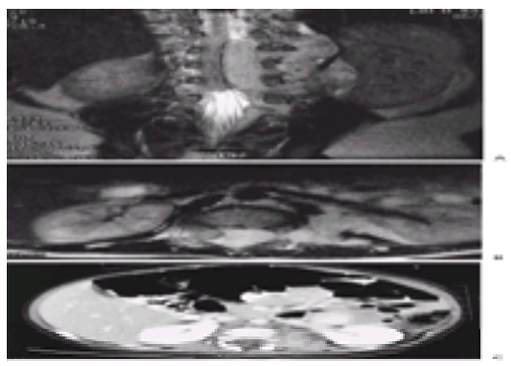

**FIGURE 9-6.** Left paraspinal neuroblastoma invading the spinal canal through several thoracic and lumbar neural foramina and displacing the thecal sac rightward as depicted on **(A)** T2-weighted coronal and **(B)** T2-weighted axial magnetic resonance images and on **(C)** axial computed tomography scanning performed with oral and intravenous but without intrathecal contrast.

## Angiography and Lymphangiography

Although invasive vascular imaging is currently performed in the context of image-guided interventional procedures, such as tumor embolization and intraarterial chemotherapy delivery, invasive vascular imaging for purely diagnostic purposes has been largely supplanted by the newer imaging modalities and is currently rarely indicated. Inferior vena cavography was initially replaced by lymphangiography and subsequently by CT and MR for evaluation of retroperitoneal lymphadenopathy, and by ultrasound, CT, and MR for the evaluation of intravascular spread of malignancy as in cases of Wilms' tumor [8] and hepatoblastoma. Although angiography is occasionally performed to evaluate tumor vasculature, particularly in some institutions when partial hepatectomy or limb salvage procedures are contemplated, most tumors, including hepatic neoplasms, can be adequately studied by noninvasive cross-sectional imaging. Although its spatial resolution is less than that of CT, MR provides a generally superior depiction of vascular anatomy. [9,10]

Invasive diagnostic neuroangiography for the evaluation of tumors has also been superseded by CT and MR. Invasive arteriography, however, is still the best way to evaluate small-vessel disease within the central nervous system (CNS), an uncommon problem in children that nonetheless can occur as a rare complication of antitumor therapy.

Lymphangiography remains a somewhat controversial issue in the diagnostic evaluation of patients with lymphoma ( Table 9-2).[11] The procedure is technically difficult in inexperienced hands, invasive, expensive, has a small but definite incidence of complications, and usually requires deep sedation or general anesthesia of preadolescent children. On the other hand, it is the only imaging procedure that allows evaluation of intrinsic lymph node architecture ( Fig. 9-7) and has been performed successfully in children as young as 14 months of age by experienced personnel. [12] Body CT, when performed with appropriate use of intravenous and oral contrast agents, is capable of identifying lymph nodes larger than a few millimeters in diameter. However, CT is not able to determine whether a lymph node is enlarged because of tumor involvement or because of reactive hyperplasia. Because reactive hyperplasia is present in 12% of children younger than 16 years of age and 19% of children younger than the age of 11 years, [13] the specificity of CT is low. The accuracy of lymphangiography approaches 95%. [14] Nonetheless, CT evaluation of the retroperitoneal lymph nodes has replaced lymphangiography in most institutions, and in those few institutions where lymphangiography is still performed, it is often reserved for patients with negative CT scans who will not receive chemotherapy. MR can demonstrate retroperitoneal lymph nodes but, like CT, cannot, at least at this time, differentiate reactive hyperplasia from malignant disease. Measurement of relaxation times and *in vivo* MR spectroscopy (MRS) (or both) may in the future, however, enable this procedure to definitively identify malignant lymph node disease.

| | Lymph-angiography | Computed tomography | Ultra-sound | Magnetic resonance |
|---|---|---|---|---|
| Accuracy | 4 | 3 | 2 | 3 |
| Technical skill required | 4 | 1 | 3 | 2 |
| Invasiveness | 4 | 1–2 | 0 | 0–1 |
| Potential complications | 3 | 1–2 | 0 | 0–1 |
| Cost | 4 | 2–3 | 1 | 3–4 |

*On a scale of 1 to 4, 4 is the highest value.
Notes: The invasiveness, potential for complications, and cost of computed tomography and magnetic resonance vary with the need for sedation.

**TABLE 9-2. RETROPERITONEAL LYMPHADENOPATHY: COMPARISON OF UTILITY OF IMAGING PROCEDURES ON A SCALE OF 1 TO 4[a]**

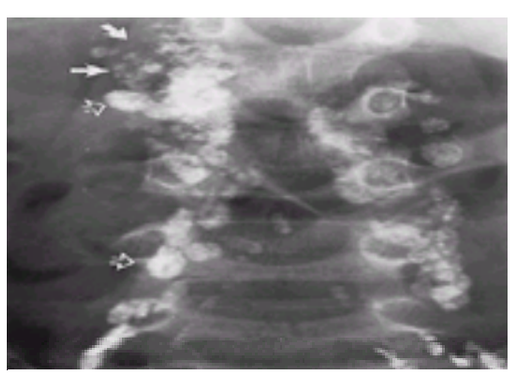

**FIGURE 9-7.** Hodgkin's disease. Bipedal lymphangiogram in 11-year-old boy demonstrates areas of tumor involvement ( *closed arrows*) and reactive hyperplasia (*open arrows*) documented at staging laparotomy. The nontumorous nodes are at least as large as the involved nodes, making differentiation by computed tomography, ultrasound, or magnetic resonance techniques difficult.

**Nuclear Medicine**

Nuclear medicine studies, especially when performed by imaging specialists with a particular interest in and experience with pediatric diseases, can be an effective diagnostic tool. Although they yield considerably less morphologic data than do other advanced imaging procedures, they can provide information regarding the metabolic status of pediatric tumors that is not available by others means.

Bone scanning is of less value than CT or MR in the evaluation of extent of involvement by primary bone tumors, but, as stated above, with the exception of Langerhans' cell histiocytosis and neuroblastoma in which the examination is commonly supplemented by radiographic skeletal surveys, bone scintigraphy is the screening examination of choice for the identification of bony metastatic disease. [15] Positive bone scans can, however, result from abnormalities other than metastases, and typically any area that is positive on bone scan should undergo radiography for further evaluation.

Liver scans are generally not as sensitive as ultrasound or CT for the detection of intrahepatic metastases or abscesses. Infiltrating diseases, such as leukemia and lymphoma, can however occasionally be better defined by radioisotope liver scans than by other imaging modalities.

Gallium-67 scanning is frequently used in the detection of infections and tumors. In children, it has been most useful in detecting rhabdomyosarcoma and lymphoma (Fig. 9-8).[16,17] In some institutions, thallium-201 has been used in the diagnosis and management of patients with brain tumors, osteogenic and Ewing's sarcoma, lymphoma, rhabdomyosarcoma, and germ cell neoplasms.[18,19]

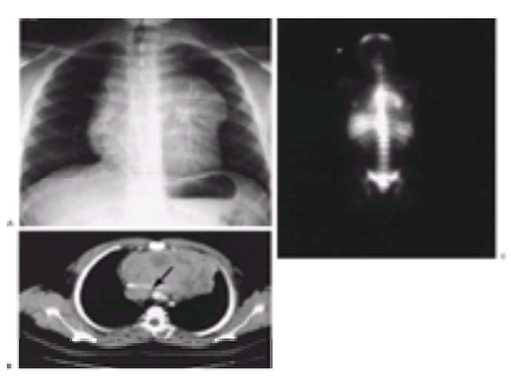

**FIGURE 9-8.** Hodgkin's disease. **A:** Posteroanterior chest radiograph shows a large mediastinal mass. **B:** Intravenous contrast–enhanced axial chest computed tomography scan confirms the presence of the mass and demonstrates compression of the left brachiocephalic vein by the tumor ( *arrow*). **C:** Gallium-67 citrate total body imaging shows physiologic tracer uptake by the liver, spleen, and bone marrow, as well as uptake by the mass.

As a further aid to the detection of focal infectious processes in the immunocompromised patient, scans using indium-111– and technetium-99m–labeled white cells have shown an ability to localize involved organs. For children, an accuracy of 86% has been reported for indium-labeled white cell imaging. [20] Although as many as one-third of patients with occult infections may show false-negative results, the lack of false positives makes a positive scan a reliable indication of a focal infectious process.[21] It may be necessary to follow labeled white cell scintigraphy with ultrasound, CT, or MR to better define a lesion, but the white cell scan offers the advantage of imaging the entire body at a lesser cost than is possible with other modalities.

The use of iodine-131 metaiodobenzylguanidine (MIBG) as a radioisotope marker in neuroblastoma is finding more adherents despite its cost and logistical difficulties.[22,23] and [24] The agent, which is also accumulated by pheochromocytomas, has been used in neuroblastoma primarily to diagnose and localize primary, metastatic, and residual disease. The potential for targeted treatment of neuroblastoma with a therapeutic doses of radiolabeled MIBG is under investigation.

Nuclear imaging has been widely used to monitor cardiac function in patients receiving cardiotoxic chemotherapy.

Recent advances in nuclear medicine imaging have been highly dependent on computer-aided technology. Single-photon emission CT uses the same computationally intense mathematical algorithms as are used in radiographic CT scanning to produce tomographic reconstructions of gamma emission data. Positron emission tomography (PET) uses so-called “coincidence imaging” to document the distribution of positron-emitting isotopes within the body and has significant potential for the depiction of physiologic and metabolic activity not evaluable by more traditional scintigraphic studies. [25] Especially useful has been the ability of PET imaging to distinguish metabolically active and therefore viable tumor from necrotic neoplasm and scar tissue. Labeling of tumor-avid agents with positron emitters may in the future permit localization of neoplastic tissue using the technique. Initially, the availability of PET imaging was significantly limited by the fact that as a research tool there was no financial reimbursement provided for PET studies and the fact that an expensive dedicated PET camera was required for imaging.

Currently, PET studies are, in many instances, reimbursed by private insurance, Medicare, and Medicaid. In addition, the cost of dedicated PET units has decreased considerably. There are also units available that can be used for both general-purpose nuclear medicine imaging and PET imaging and, in some cases, also CT scanning. An ongoing difficulty with PET imaging, however, arises from the fact that most positron emitters require a cyclotron for their production, an expensive proposition, and that most have quite short, on the order of seconds or minutes, half-lives, necessitating that a cyclotron be sited in close proximity to the PET unit. However, fluorine-18, the most widely used positron-emitting isotope at present and the isotope used to produce labeled glucose [fluoro-2-deoxyglucose (i.e., $^{18}$F-FDG)], has a relatively long half-life of 110 minutes, permitting its production at a central site and distribution throughout a community to multiple end users, a process commonly used with nuclear pharmaceuticals. Initially, the major use of PET scanning had been in neuropsychiatric and cardiovascular studies. The technique has, however, been found to be useful in evaluating lung and colon cancer, lymphoma, melanoma, and brain tumors, and investigation of its use in the diagnosis and management of neuroblastoma, bone tumors, and rhabdomyosarcoma is ongoing.[26,27]

**Ultrasound**

Although high-frequency sound was first used for diagnostic purposes more than 30 years ago, its true value has only been realized in the past two decades with the rapid development of advanced technology.[28] The ultrasound procedure uses sound waves in the range of 1 to 20 million cycles per second (1 to 20 MHz), well above the 20,000 Hz that is the upper limit of aural perception by human beings. Higher frequency sound permits greater imaging resolution but penetrates tissues to a lesser depth than does lower frequency sound. Thus, high-frequency transducers are used for high-resolution imaging of superficial structures such as the skin, testes, thyroid gland, and breast tissues. Lower frequency transducers are used for imaging of the intraabdominal organs. The sound waves are generated by a handheld transducer that contains a piezoelectric crystal. This crystal vibrates when an electric current is passed through it, propagating sound waves at a specific frequency. The sound can be directed toward a particular body part and can be focused to greater or lesser degrees depending on technologic factors. The transducer's crystal also acts as a receiver for sound waves reflected from internal structures. The vibrations generated in the crystal by these echoes produce electrical signals that can be digitized, processed, and displayed, typically in real time on a video monitor. The number and strength of the echoes depends on the quantity and nature of tissue interfaces within the insonated structure. Because there are no interfaces in a fluid-filled cyst, for instance, no echoes within it are seen, and the structure is known as *sonolucent* or *anechoic*. Tissue interfaces, such as those found in solid tumors, produce echoes of variable number and intensity. The resulting image is described as *echogenic*. Interfaces between air and soft tissues produce such strong echoes that depiction of anatomy beyond such interfaces is typically not possible. Bone attenuates sound waves to such a degree that anatomic detail beyond it is largely obscured.

Ultrasound examination, particularly when performed with the newest computerized equipment, is an excellent tool in the evaluation of the abdomen, pelvis, thyroid, breasts, and scrotal contents of children. Its utility in examining other areas of the body, such as the chest and CNS, depends on the amount of bone or gas present in or near the structure to be imaged. Because ultrasound examinations are relatively quick and inexpensive, use no ionizing radiation, have no known complications or side effects, and usually do not require sedation or anesthesia, they represent an ideal screening examination of the abdomen after plain film imaging. Technically successful ultrasound examinations are easier to perform in infants and young children than in older children and adults because of the relative paucity of abdominal fat in younger patients.

Ultrasound is particularly useful in evaluation of the liver, the gallbladder, the spleen, the kidneys, and the pelvic organs. In children, the pancreas, the aorta, and the inferior vena cava are typically well imaged. Retroperitoneal lymphadenopathy can frequently be identified but not as routinely as with CT. Intraperitoneal abnormalities may be difficult to image, especially if small, because of the presence of intraluminal intestinal gas. Ultrasound can easily and accurately differentiate solid from fluid-filled masses and can usually distinguish the organ of origin of an abdominal mass ( Fig. 9-9). During open surgery, high-resolution sonographic imaging can be performed by placing a sterile dressed ultrasound probe in direct contact with the surface of an organ of interest. Such intraoperative ultrasound has proven helpful in localizing tumors within the brain, spinal cord, pancreas, liver, and kidneys that are inapparent to physical inspection.

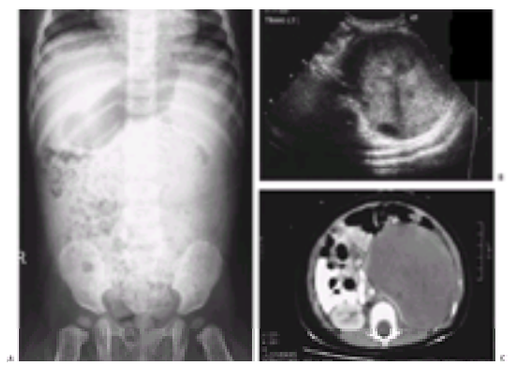

**FIGURE 9-9.** Wilms' tumor. **A:** Supine abdominal radiograph view demonstrates mass effect in the left side of the abdomen. **B:** Ultrasound confirms the presence of a solid left-sided tumor of renal origin. **C:** Axial computed tomography scan with oral and intravenous contrast shows the mass with a slender rim of enhancing renal tissue at its posterior margin.

The major disadvantages of ultrasound are that the resolution is poor compared with CT or MR; the examination depends on the skill, experience, and expertise of the sonographer; and the presence of bone or gas interferes with image production, limiting its utility in many anatomic areas.

Technologic advances in ultrasound have led to improved image resolution and greater diagnostic capability. Doppler imaging for blood flow improved markedly in the early 1990s, and, although the utility of Doppler waveform analysis in distinguishing malignant from benign disease has been limited, the ability of the method to detect intravascular thrombus, both malignant and benign, has proven to be quite valuable in tumor staging, [29] in assessing for complications of thrombogenic chemotherapeutic agents, and in providing guidance for placement of central lines.

**Computed Tomography**

A CT image represents a display of a thin section of anatomy. However, rather than being produced as are conventional plain films by radiation from a stationary x-ray tube striking a piece of film after passing through the patient, with CT the x-ray source is a fan-shaped beam produced by an x-ray tube that rotates 360 degrees around the body part being examined. Instead of film, the x-rays strike a series of small detectors that convert the energy into electrical signals. These thousands of signals are fed into a computer, which analyzes and constructs an image that can be displayed on the computer console. By changing various settings on the console, the radiologist can manipulate the image to bring out those features of particular interest. For instance, the images can be viewed to highlight the details of the highly radiodense bones or the largely radiolucent lungs. The image can be photographed on film for interpretation and storage or can be archived on tape or disc and accessed electronically.

The patient is placed on a movable table and the body part to be examined is positioned within the gantry of the CT unit, a structure that looks like an elongated doughnut. Because the geometry of satisfactory image production necessitates a relatively small diameter for the gantry, most scanning must be performed in transaxial cross-section, the images being oriented perpendicular to the long axis of the body.

Studies performed with iodinated intravascular contrast are typically more informative than those performed without such contrast. In the head, intravenous contrast material typically increases the conspicuity of brain pathology be it neoplastic, inflammatory, or ischemic in nature and, in the case of tumors, is particularly helpful in differentiating neoplastic tissue from surrounding edema. In the body, intravenous contrast is useful for optimal assessment of the parenchyma of the liver, spleen, kidneys, and adrenal glands, evaluation for intratumoral necrosis, and differentiation of vascular from nonvascular structures, including lymph nodes ( Fig. 9-10). Although there is a small but definite incidence of morbid and even fatal reactions to traditional hyperosmolar ionic iodinated contrast material among adults, the incidence of such reactions is so low in children that ionic material can be used with a high degree of safety in the pediatric population. [30] Nevertheless, the increased safety of non-ionic, low-osmolar contrast materials has persuaded most pediatric radiologists to use them despite the considerably greater financial cost of the non-ionic agents. The primary concern about the use of intravenous contrast material in children is potential compromise of renal function; this can be obviated by having patients well hydrated before the procedure and maintaining the total intravenous dose of iodinated contrast material generally at a level not greater than 2 to 3 mL per kg of body weight, with a maximum dose of 250 to 300 mL. (Of note, although such high total doses may be necessary for angiography, most CT

examinations, even in large patients, can be accomplished with less than 200 mL of contrast.) Iodinated contrast material is relatively but not absolutely contraindicated in patients with anuria, renal failure, combined liver and hepatic dysfunction, severe thyrotoxicosis, myeloma, and suspected or known pheochromocytoma.

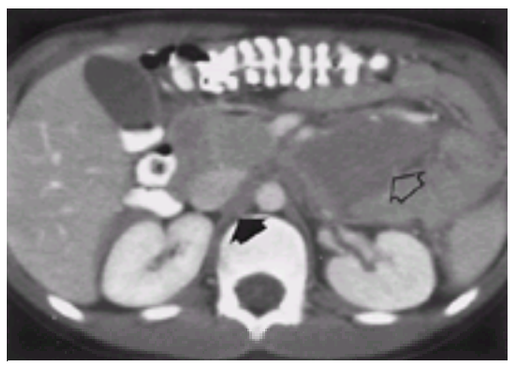

**FIGURE 9-10.** Burkitt's lymphoma. Scanning electron beam computed tomography demonstrates mesenteric tumor (*open arrow*) and also retroperitoneal lymphadenopathy (*solid arrow*), which is easily distinguished from the contrast-enhanced inferior vena cava anterior and to the right of the tumor and the aorta to the left of the tumor.

Appropriate use of oral contrast material in abdominal and pelvic scanning is critical to differentiate intestinal loops from lymph nodes and soft tissue masses. In children, the use of such material is especially important because of the lack of retroperitoneal and mesenteric fat that in adults surrounds and separates structures, increasing their conspicuity.[31]

Disadvantages of CT include its cost, its relatively high radiation dose, and the need for conscious sedation or general anesthesia in the young patient. Initially, one of the additional disadvantages of CT was its inability to produce images in planes other than the transaxial one. Advances in computer technology, however, now permit the production of computer-reconstructed images in any plane desired. Reconstructing the CT data, however, is cumbersome, and the resulting images are prone to misregistration artifacts caused by patient motion; thus, the multiplanar imaging capability of CT is not quite so robust as that of ultrasound and MR imaging. Three-dimensional reconstruction of CT images is also possible, but this method is not commonly used in the evaluation of oncologic patients, except those with head and neck tumors.[32]

High-resolution CT (HRCT) has greatly enhanced the ability of radiologists to identify interstitial lung disease so mild as to be inapparent on conventional chest radiographs and CT scans.[33] For HRCT, 1-mm thick sections are obtained using the bone reconstruction algorithm on the CT computer, a technique that accentuates parenchymal detail. The major value of HRCT in pediatric oncology is the identification of pulmonary Langerhans' cell histiocytosis ( Fig. 9-11)[34] and interstitial lung disease produced by chemotherapeutic agents. Among other uses, HRCT has also been employed in the evaluation of febrile neutropenic patients and in bone marrow and blood stem cell transplant recipients to evaluate for pneumonias occult to conventional radiography. [35]

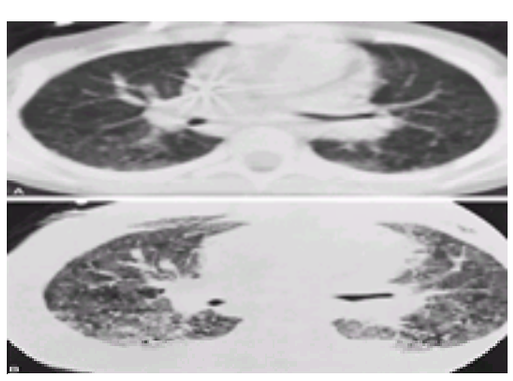

**FIGURE 9-11.** Langerhans' cell histiocytosis. Conventional **(A)** and high-resolution **(B)** computed tomography scans demonstrate diffuse interstitial lung disease with characteristic focal emphysematous changes (i.e., black holes in lungs), seen to better advantage on high-resolution computed tomography.

Spiral CT, also known as *helical* or *volumetric acquisition CT*, has decreased scanning time, reduced need for sedation, and improved resolution. [36] With conventional CT imaging, the CT table moves stepwise 1 to 20 mm at a time, and a CT slice is obtained at each table position. With helical scanning, both table motion and data acquisition are continuous during the course of the examination. Because the resulting helical data is acquired in a volumetric fashion, rather than slice by slice as in conventional CT, there are no “geographic misses” because of small lesions falling between slices. Three-dimensional reconstruction can also be performed with far greater detail than was possible with conventional CT. [37] Compared with conventional CT, spiral imaging can be accomplished more rapidly, at lesser radiation doses, and with smaller volumes of intravenous contrast. The speed advantage of spiral units has been augmented with the recent introduction of multislice CT units that use, at present, up to four detector arrays that simultaneously collect data from multiple locations along the axis of rotation with a gantry that rotates up to twice as fast as traditional gantries.[38,39]

Electron beam CT was initially introduced as a tool for cardiovascular imaging [40] but has proved useful in generalized body imaging as well. Rather than moving the x-ray source and x-ray detectors about the patient, as is done in conventional CT, an electromagnetically steered electron beam is used to generate x-rays that, like those propagated by the rotating source used in conventional CT, strike the patient from all angles, permitting tomographic reconstruction of radiographic detail ( Fig. 9-10). The method permits very rapid scanning even exceeding that possible with multislice helical units, a feature, which though it is of limited consequence for most indications, is helpful in imaging rapidly moving structures such as the lungs and especially the heart. The units are, however, costly, the advantages of the increased speed of electron beam scanning limited, and the imaging of the brain less satisfactory than that obtainable using conventional scanners; consequently, there are relatively few electron beam CT units in use.

The advantages of CT in evaluation of the cranial contents were apparent immediately after its introduction in 1972. Pneumoencephalography has disappeared from the armamentarium of the neuroradiologist, and intracranial arteriography is rarely used for pediatric patients with tumors. As the equipment has become technologically more sophisticated over the past 20 years, speed of imaging and image resolution have improved dramatically, allowing rapid identification of tumor masses, intracranial calcifications, hydrocephalus, and cerebral and cerebellar complications of anticancer therapy. Although MR has supplanted CT as the imaging modality of choice for detailed evaluation of the brain, CT imaging, because it is quicker, less expensive, and more readily available than MR and highly informative, continues to play a major role in the evaluation of intracranial pathology. Although CT, especially after administration of intrathecal contrast, was at one time an important modality for evaluation of the contents of the spinal canal, such evaluations are now almost exclusively performed with MR. MR has also superseded CT in the evaluation of bone tumors and soft tissue masses within the extremities, and within the chest, abdominal, and pelvic walls. Although MR has made inroads, CT remains the primary advanced imaging modality for studying the contents of the thoracic, abdominal, and pelvic cavities, except for the uterus and ovaries, which are optimally evaluated by a combination of ultrasound and MR imaging.

**Magnetic Resonance Imaging**

The role of MR in the evaluation of pediatric oncologic disease has expanded at a rapid pace over the last 15 years. Based on the concept of nuclear MR first described by Bloch[41] and Purcell,[42] clinical MR is based on “alignment” of the body's hydrogen nuclei in a strong, uniform magnetic field. Once aligned, these hydrogen nuclei precess or “wobble” at a frequency proportional to the magnetic field strength. When these hydrogen nuclei are bombarded with radiofrequency (RF)

waves at the precessional frequency, energy is absorbed by the nuclei. The radio waves are then turned off, and the hydrogen nuclei return to their initial alignment, releasing energy that induces voltage in a wire receiving coil. As with CT, MR images represent “slices” through the body that are made up of a specified number of volume elements, called *voxels*. Unlike CT, in which the “slices” are almost always by necessity obtained in the transaxial plane, MR imaging can be performed in any desired plane. Using the same mathematical construct, the Fourier transformation, used in CT and single-photon emission CT imaging, each MR voxel is assigned a shade of gray between white and black that reflects the amount of hydrogen and the rate at which equilibrium is reestablished with the magnetic field after RF excitation.

Three classes of magnets, permanent, resistive, and superconducting, are used for MR. The field strengths used in clinical imaging range from low (0.15 T) to medium (0.35 to 0.50 T) to high (1.5 to 3.0 T) field strengths. High field strength systems have the potential advantage of enhanced signal-to-noise ratio and therefore improved image quality, as well as the potential for MRS. Disadvantages of the high field strength systems include the need for greater shielding (which significantly increases siting costs), significant fringe fields (and therefore an increased risk for attractive force-induced accidents), and an increased potential for biologic hazard arising from the need for higher RF pulse strengths. A less hazardous environment, without significant fringe fields, is afforded by the lower field strength magnets. At lower field strengths, ferromagnetic items (including anesthesia equipment) can be used in the imaging suite without the risks involved at higher field strengths. High field systems require that the imaged body part be placed within a narrow tunnel like bore in the magnet, a condition that can preclude imaging of very large patients or of claustrophobic individuals. Lower fields can be generated with so-called “open” systems in which the imaged body part is placed between two parallel plates that are far less confining than the bore of a high field magnet. [43,44]

A variety of RF coils are used in MR. The shape of the coil, the type of wire used to form it, the number of wire turns, the net capacitance of the coil, and many other factors influence the performance. The coils are designed to maximize their response for the body part studied at the precessional frequency of the spins, thus optimizing the signal-to-noise ratio. [45]

A detailed explanation of the multiple MR sequences available for clinical use is beyond the scope of this book; the reader is referred to one of several excellent texts describing in detail the various MR sequences. [45,46 and 47] In the most commonly used sequence, called *spin echo*, the time between the subsequent RF pulses [i.e., repetition time (TR)] and the time between the initial pulse and data collection [i.e., echo time (TE)] can be manipulated so that a T1-weighted or a T2-weighted image is acquired. An image acquired with a short TR (300 to 600 msec) and short TE (10 to 20 msec) emphasizes the T1 characteristics of the tissue, and an image acquired with a longer TR (>2,500 msec) and a longer TE (>80 msec) reflects primarily the T2 characteristics of the tissue. The T1 and T2 characteristics of tissue reflect the inherent tissue relaxation times, which are exponential time constants that describe the realignment of the tissue protons with the main magnetic field after cessation of the RF pulse. The T1 relaxation time, or longitudinal relaxation time, describes the return of the signal to equilibrium (i.e., alignment with the main magnetic field); the T2 relaxation time, or transverse relaxation time, describes the exponential loss of alignment or phase coherence of the magnetization of the protons. On T1-weighted images, tissues with short T1 relaxation times (e.g., fat) are seen as bright signal intensity, and tissues with longer T1 relaxation times (e.g., water, tumor) are seen as intermediate to low signal intensity. On T2-weighted images, tissues with short T2 relaxation times (e.g., muscle, tendon) are seen as low to intermediate signal intensity, and tissues with longer T2 relaxation times (e.g., water, tumor) are seen as bright signal intensity. Images acquired with TRs and TEs that are intermediate to those used to acquire T1- and T2-weighted images are often referred to as *proton-density images*. The signal characteristics on these images reflect a complex mixture of T1 and T2 relaxation times and the proton density of the imaged tissue.

New sequences seem to appear in every issue of imaging journals. These sequences have focussed primarily on reducing scanning time, suppressing fat signal for better evaluation of lesions, and increasing conspicuity of blood flow. The latter have been so successful that a new subfield of MR imaging, MR angiography, has been created that, in the realm of pediatric oncology, has been particularly useful in the presurgical evaluation of liver tumors and of bone and soft tissue sarcomas and in assessing the central venous system before central line placement (Fig. 9-12). [48,49 and 50] In neuroimaging, considerable attention has been directed recently to diffusion imaging, a technique that characterizes tissues on the basis of the molecular motion of constituent water and which is highly sensitive for the detection of acute ischemia. [51,52]

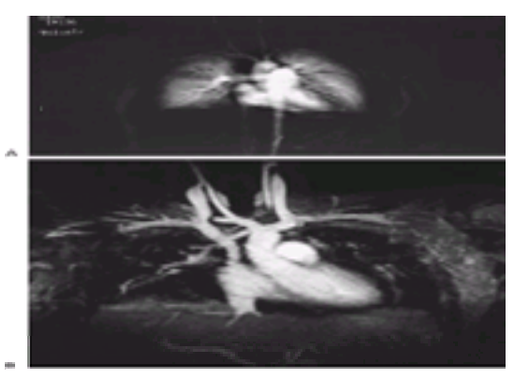

**FIGURE 9-12.** Normal magnetic resonance (MR) angiogram performed before central venous line placement in a patient who had undergone bone marrow transplant for myelodysplastic syndrome after treatment of lymphoma. **A:** Arterial phase contrast-enhanced MR angiogram shows the pulmonary arterial tree, the aorta, and the great arteries well. **B:** More delayed imaging shows both arterial and venous structures and demonstrates patency of the superior vena cava, and the innominate, subclavian, and internal jugular veins.

MR offers several advantages over CT. MR delineates normal soft tissues and discriminates abnormal from normal soft tissues better than CT and does so without the use of ionizing radiation. Iodinated contrast agents are not required, beam-hardening artifacts from bone are eliminated, and imaging can be performed in any plane desired. Disadvantages of MR include its relatively great cost and limited availability as well as its limited ability to detect calcification and to assess the pulmonary parenchyma and the bony cortices. The absence of a widely accepted GI contrast medium impedes the ability of the test to discriminate between normal bowel and intraperitoneal pathology. Finally, MR image acquisition is slower than image acquisition by ultrasound, CT, or conventional radiography, a notably unfortunate feature of the technique as MR images are also highly susceptible to degradation by normal cardiac and respiratory motion, bowel peristalsis, and vascular pulsations. In addition, gross patient motion compromises MR imaging substantially, and, as a consequence, children who would not require sedation for other imaging modalities, including CT, often require sedation for MR imaging.

MR is superior to CT in diagnosing most instances of intracranial tumors, especially in the posterior fossa ( Fig. 9-13). The spinal cord and its coverings, the surrounding intracanalicular soft tissues, the cerebrospinal fluid, and the vertebrae can all be studied without the need for myelography. Evaluation of direct coronal, sagittal, oblique, and transverse images allows more precise localization of neoplastic disease.

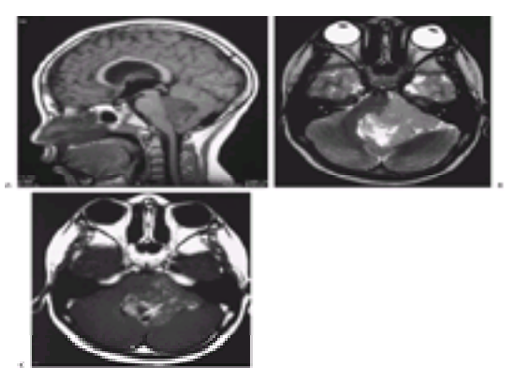

**FIGURE 9-13.** Malignant ependymoma. T1-weighted sagittal **(A)** and T2-weighted axial **(B)** magnetic resonance images demonstrate a mass within the fourth ventricle that extends left laterally through the foramen of Luschka into the left cerebello-pontine angle. **C:** T1-weighted gadolinium-enhanced image demonstrates some irregular enhancement within the lesion.

The calcification that occasionally accompanies pediatric oncologic disease, including brain tumors, has a variable appearance on MR. Although heavily calcified regions can be seen as low signal intensity on T1- and T2-weighted images, calcium that is embedded in a protein matrix or that has a speckled appearance on conventional radiography is often inapparent. The small bones of the skull and face are also often not adequately evaluated by MR. Therefore, if the identification of calcium or the precise evaluation of the small bones of the face and skull is required, CT is recommended in addition to or in place of MR. Conventional radiographs can also on occasion be helpful, especially in evaluating the facial bones and the skull.

Application of MR studies to extracranial structures has advanced rapidly. Although MR is equal to or better than CT in the anatomic definition of many mediastinal [53,54] and intraabdominal tumors,[55,56] it is the direct acquisition of images in the coronal and sagittal planes and the ability to visualize flowing blood in vessels without the need for intravenous contrast that promise an increasing role for MR in the evaluation of extracranial tumors.

MR is thought to be generally superior to CT in the evaluation and staging of musculoskeletal tumors, except in the detection of calcification, early cortical bone erosion, and the identification of periosteal reaction. [57,58,59] and [60] However, as knowledge of the MR appearance of these entities increases, examiners are finding MR equal, and in some cases superior, to CT in these aspects of musculoskeletal tumor evaluation ( Fig. 9-5). The increased accuracy of MR compared with CT in the evaluation of musculoskeletal tumors is particularly important when primary radiation therapy or limb-salvage procedures, or both, are being considered.

One of the difficulties when using MR in the evaluation of musculoskeletal lesions is the delineation of extraosseous soft tissue masses from the edema that surrounds them, as both appear as intermediate signal intensity on T1-weighted images and increased signal intensity on T2-weighted images. When present, soft tissue edema may not be as bright as soft tissue tumor on T2-weighted images and typically has a feathery appearance conforming to the local soft tissue planes. Adding to the confusion is the as yet unresolved question of whether to extend radiotherapy fields to encompass regions of suspected peritumoral edema. Intramedullary tumor is not typically associated with significant surrounding edema so that marrow space signal abnormalities detected by MR correlate closely with the gross extent of tumor at surgical resection, usually within 3 to 4 mm.

There continues to be significant progress in the use of MR for the evaluation of pediatric marrow disease. A statistically significant prolongation of T1 relaxation time in diseased vertebral marrow was first reported in 1986. This allowed differentiation of leukemic from normal marrow based solely on the comparison of T1 relaxation times (Fig. 9-14).[61] Further work using bulk T1 relaxation times[62] and chemical shift imaging[63] confirmed these findings. There is also a prolongation of vertebral marrow T1 relaxation times in relapsed leukemia compared with the T1 relaxation times of leukemia in remission. In several instances, the diagnosis of relapsed leukemia has been made using calculations of MR T1 relaxation times several weeks before the diagnosis was appreciable by bone marrow aspirate or biopsy. There are several reasons for this phenomenon, including the fact that vertebral marrow, as the primary site of hematopoiesis in children, may reflect the changes of leukemia earlier than iliac crest marrow. In addition, iliac marrow sampling error could account for some initially false-negative results. Although an initial bone marrow biopsy is still required for children presenting with new-onset leukemia, MR may ultimately obviate the need for bone marrow aspirates or biopsies during routine follow-up. MR of the marrow is also useful in the evaluation and detection of focal areas of macroscopic lymphoma in patients who are symptomatic but have negative bone scans.[64,65]

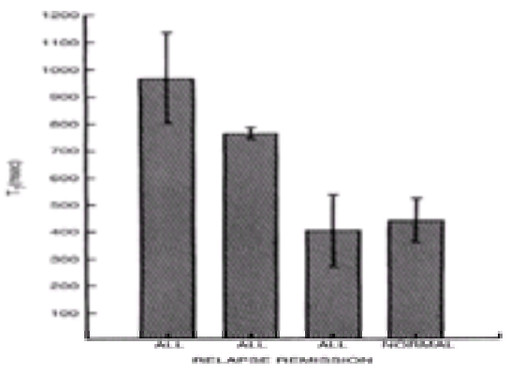

**FIGURE 9-14.** Marrow T1 relaxation times in leukemia. The histogram shows mean T1 relaxation times of the vertebral marrow for each of four groups: newly diagnosed acute lymphocytic leukemia (ALL), leukemia in relapse, leukemia in remission, and normal age-matched controls. The T1 relaxation time is significantly prolonged for children with disease compared with age-matched controls with no evidence of active disease. The standard deviation for each measurement is shown. (From Moore SG, Gooding CA, Brasch RC, et al. Bone marrow in children with acute lymphocytic leukemia: MR relaxation times. Radiology 1986;160:237.)

Despite the fact that the superior soft tissue contrast differentiation of MR permits identification of most tumors without the administration of any intravenous contrast agent and the fact that flowing blood can also be readily identified without resort to contrast administration, intravenously administered gadolinium-containing agents are used in MR much like intravenously administered iodinated contrast in CT to better assess tumor vascularity and endothelial permeability and to evaluate for intratumoral necrosis.[66] The dynamics of contrast uptake by tumors is under current investigation with the belief that a determination of the rate of contrast uptake will be helpful in predicting and documenting a given tumor's response to therapy. [67,68] Recent studies, for example, suggest that in osteosarcoma rapid and pronounced access of contrast to the extravascular space on initial pretherapy dynamic-enhanced MR imaging (DEMRI), a considerable decrease in this access as a consequence of therapy and minimal access at the conclusion of therapy confer a good prognosis. [69] In addition to intravenously administered gadolinium, other MR contrast agents include intravenously administered iron-containing compounds for liver, spleen, and bone marrow imaging, as well as a variety of orally administered materials to manipulate the signal from the GI tract, including perfluoro-octylbromide (PFOB) and agents containing barium, iron, manganese, and lipid.

*In vivo*, MRS remains a promising but unproven clinical tool. A variety of technical and physiologic factors have impeded the transfer of MRS from the laboratory to the clinic, but several major institutions have made major commitments to MRS research, which should bear fruit shortly. [70,71] Investigators have focused their attentions on proton (H) and phosphorous (P31) magnetic spectroscopic imaging (MRSI).[72] H-MRSI of cerebral neoplasms, a technique that can determine the relative concentrations of choline and *N*-acetyl-aspartate within tissues, appears to be particularly promising in predicting outcome for patients with a variety of brain tumors. [73]

There are few risks involved in MR imaging. Although there are no known adverse biologic effects on human subjects from static magnetic fields of the strengths in clinical use,[74] a number of known bioeffects may potentially cause physiologic changes at higher field strengths greater than 2 T. These result primarily from the RF and rapidly changing magnetic field gradients that are used to spatially encode the image data. This exposure to RF fields results in the generation of heat and the formation of electric current within the body tissues. Large metallic implants may absorb RF energy and heat the local area, but the temperature elevation is minimal, and the heating of these metallic implants is thought to be of no clinical consequence. [75] Ferromagnetic metals, such as iron, copper, cobalt, type 400 stainless steel, and nickel, are more likely to interact with the magnetic field than nonmagnetic materials, such as silver, tantalum, and type 300 stainless steel. Nonmagnetic material is used in orthopedic devices, surgical clips, and suture material. The interaction of ferromagnetic objects with the magnetic field can result in movement or torque of the object, induction of electric currents, heating, and MR signal distortion. Contraindications to MR therefore include exposure to ferromagnetic particles, particularly when these particles may be lodged within the eyes or CNS. In general, there is no contraindication to imaging a child with intraabdominal surgical clips. Many of the older intracranial aneurysm clips are ferromagnetic, and imaging of patients with such ferromagnetic clips is contraindicated. Most prosthetic heart valves manufactured after 1964 are not ferromagnetic, but consultation with the radiologist is recommended before ordering an MR examination for a child with a prosthetic heart valve. Most stainless steel orthopedic implants, including those used for scoliosis and internal fixation, are not significantly affected by imaging at the field strengths in clinical use. However, these nonferromagnetic metals can occasionally distort the MR image.

Cardiac pacemakers are affected by induced current caused by the changing field gradients and by the motion of the patient as she or he enters and leaves the static magnetic field. Because this can result in damage or malfunction of the pacemaker, MR examination of these patients is not recommended. As long-term studies of the effects of MR imaging on human fetuses are not available, scanning of pregnant women should be undertaken with caution. Nevertheless, MR imaging of pregnant women has been performed without apparent detriment to the mother or the fetus, and the technique has in fact been advocated for the prenatal assessment

of complex fetal anomalies inadequately elucidated by ultrasound.

The role of MR in the evaluation of pediatric malignancy has evolved rapidly. MR is unequivocally the imaging study of choice for CNS tumors, [76] musculoskeletal tumors,[77] and lesions of the chest and abdominal and pelvic walls. It is complementary to ultrasound in the assessment of the female genital tract and is currently being widely investigated for the evaluation of intrathoracic, intraabdominal, and intrapelvic malignancies.

### Interventional Radiology

During the past decade, image guided interventions have played an increasing role in the diagnosis and therapy of children with cancer. Needle biopsies of various organs under fluoroscopic observation have been done for some years, but the development of ultrasound, CT, and, most recently, MR guidance for needle biopsy has greatly increased the utility of this procedure. Recently developed ultrafast CT and MR techniques, so-called CT and MR "fluoroscopy," have further facilitated image-guided intervention. Lesions in the chest, liver, retroperitoneum, abdomen, and bone are readily accessible to biopsy procedures with appropriate imaging guidance (Fig. 9-15).[78,79,80 and 81] Diagnostic accuracy of 95% has been reported.[82] In experienced hands, the procedures are accurate and safe, although they may require anesthesia in young children. Because small-bore needles are frequently used, cytologic examination by an experienced pathologist is typically necessary to determine whether a biopsied lesion is benign or malignant. Often, the precise nature of a malignancy cannot be diagnosed, and for this reason thin-needle biopsies have been more useful for the determination of recurrent disease than for making a specific primary diagnosis. If a specific histologic diagnosis is needed from a percutaneous biopsy, larger-bore aspiration needles or cutting needles can be used. However, the use of these needles entails a higher risk of complications, especially post-biopsy bleeding. Patients undergoing such procedures often undergo a preparatory screen of clotting function, and these procedures, like all other image-guided interventions, should be performed by an experienced radiologist capable of treating potential complications.

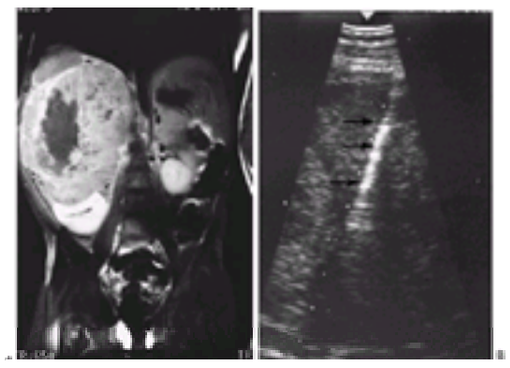

**FIGURE 9-15.** Neuroblastoma. **A:** Coronal fat-saturated, gadolinium-enhanced, T1-weighted magnetic resonance image demonstrates a centrally necrotic mass above the right kidney. **B:** Percutaneous biopsy under ultrasound guidance yielded neuroblastoma. Note the echogenic biopsy needle within the lesion ( *arrows*). (Courtesy of Dr. Taylor Chung, Department of Diagnostic Imaging, Texas Children's Hospital, Houston, TX.)

Interventional radiologists perform vascular catheterization for infusion of chemotherapeutic agents especially in cases of hepatic malignancies and for embolization of tumors.[83,84] They can also radioablate some tumors percutaneously. In many children's centers, radiologists create gastrostomies and place central lines and enteric feeding tubes.

Interventionists have also been active in the treatment of complications of tumor and of antitumor therapies. Such treatments include, but are not limited to, relief of biliary and urinary tract obstruction, endovascular stenting, percutaneous cholecystostomy, and percutaneous abscess drainage. [85]

## GENERAL CONCEPTS OF PEDIATRICTUMOR IMAGING

### Diagnosis

Although details of the diagnostic imaging of specific tumors are discussed in subsequent chapters of this text, some basic guidelines for the imaging of children with malignancies are offered at this juncture ( Table 9-3).

**TABLE 9-3. RECOMMENDED IMAGING STRATEGIES FOR PATIENTS WITH SUSPECTED MALIGNANCIES**

#### *Central Nervous System Tumors*

Although cranial CT is an excellent tool for the evaluation of the CNS in children with neurologic symptoms, MR has become the study of choice. [86] One of the particular problems with cranial CT in children has been adequate evaluation of the posterior fossa because of the bone artifacts produced on CT scans. Because most CNS tumors in childhood are located in the posterior fossa, MR should be used because of its superior imaging capabilities for infratentorial lesions ( Fig. 9-13). Although no imaging study can replace histologic diagnosis of CNS tumors, the characteristic location and imaging features of many brain tumors in children permit reasonably reliable presurgical diagnosis. MR has also become the modality of choice for identifying lesions of the spinal cord and its coverings. [87] The need for plain film myelography and for spinal CT with intrathecal contrast material has been virtually eliminated by MR. A thorough discussion of imaging studies in the evaluation of the CNS can be found in Chapter 27.

#### *Thoracic Tumors*

Primary tumors of the bronchi and lungs, such as pulmonary blastoma and adenoma, are extremely rare in children. They are commonly evaluated by a combination of conventional radiography and CT imaging. Occasionally, MR imaging can be helpful. Most malignancies involving the pulmonary parenchyma, however, are metastatic in nature and are discussed in the portion of this chapter devoted to tumor staging.

Primary tumors of the mediastinum are best defined by the compartment in which they arise. Tumors arising in the posterior mediastinum are largely of neurogenic origin. Neuroblastoma is the most common malignant tumor of the posterior mediastinum. Erosion of ribs, widening of spinal neural foramina, and calcification of the mass are plain film signs that suggest the correct diagnosis ( Fig. 9-16). Although the differential diagnosis is extensive, the common benign lesions have radiographic features that usually differentiate them from neuroblastoma, ganglioneuroma, and ganglioneuroblastoma. Neurenteric cysts for instance are always associated with

congenital anomalies of the thoracic spine whereas neurofibromas are frequently associated with acute-angle scoliosis and multiple small paraspinous masses at several levels along the spine. Calcifications are also not present in these two benign conditions.

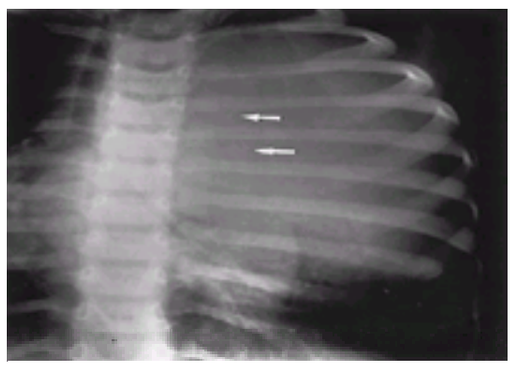

**FIGURE 9-16.** Thoracic neuroblastoma. An overpenetrated anteroposterior view of the chest demonstrates a large mediastinal mass with spreading of upper ribs, erosion of several posterior ribs, and speckled calcifications ( *arrows*).

Cross-sectional imaging is essential in the evaluation of a possible posterior mediastinal neuroblastoma. CT is typically performed, although MR yields more definitive information regarding the relationship of the mass to the neural foramina and spinal canal ( Fig. 9-6). CT can strongly suggest a diagnosis of neuroblastoma by depicting calcification within the mass (90% of neuroblastomas are calcified on CT, but only 50% are calcified on plain film radiography). [88] MR is less sensitive for the detection of intratumoral calcification than is CT. [89]

The middle mediastinum may be involved by lymphadenopathy secondary to leukemia or lymphoma, but this rarely occurs in the absence of anterior mediastinal lymphadenopathy or thymic infiltration. Acute myelogenous leukemia and chronic lymphocytic leukemia may be exceptions. Plexiform neurofibromatosis and juvenile fibromatosis may involve the middle mediastinum as a large infiltrating mass rather than the multiple paraspinous masses more typically seen in neurofibromatosis. Although histologically benign, plexiform neurofibromatosis and juvenile fibromatosis are potentially fatal because of involvement of the tracheobronchial tree or the great vessels. CT is useful for evaluation of the middle mediastinum. [89] As in any CT evaluation of the mediastinum, intravenous contrast material should always be used in an attempt to differentiate cardiovascular structures from abnormal masses. Numerous studies of MR of the mediastinum suggest that this method will likely supplant CT, [90] but CT continues to be the procedure of choice in most centers.

Evaluation of the hilar lymph nodes is particularly important in Hodgkin's disease but also may be useful for other tumors. Studies comparing MR and CT in the initial diagnosis[91] and follow-up[92] of patients with Hodgkin's disease have been performed. On MR and CT scans, lymphadenopathy is appreciated as nodal enlargement. However, no definite size criterion of nodes allows for separation of normal from abnormal mediastinal nodes in children. In adults, a lymph node exceeding 1 cm in diameter is believed to be pathologic. In infants and young children, hilar lymph nodes are not usually seen, and their presence should be considered abnormal. No signal criteria on MR examination allow accurate differentiation of benign, hyperplastic nodes from neoplastic nodes. However, MR has a distinct advantage in the evaluation of the hili because vessels can be easily distinguished from nodes without the use of intravenous contrast. Flow-sensitive cine MR studies of the mediastinum can be performed in cases of suspected compression or invasion of vessels by enlarged nodes.

The anterior mediastinum is the compartment most frequently involved by malignant disease, typically leukemia or lymphoma ( Fig. 9-8). These diseases may cause anterior mediastinal lymphadenopathy, may produce infiltration of the thymus by malignant cells, or may cause both of these processes to occur. Plain film radiographic analysis of anterior mediastinal masses in children can sometimes be difficult because of the wide range of normal thymic size. The identification of malignancy within the thymus itself is complicated by the fact that the gland can be quite big, especially in infants and young children, and be entirely normal. Also, the gland can be involved by malignancy without being strikingly enlarged. Often, the diagnosis of intrathymic neoplasm is simplified when a prominent thymus is seen in conjunction with lymphadenopathy in other areas of the mediastinum, such as the paratracheal, tracheobronchial, and hilar regions. In the absence of associated lymphadenopathy, the finding on conventional radiographs of a loss of the normal wavy contours of the thymus may suggest pathologic infiltration of the organ. Aside from potentially demonstrating heterogeneous radioattenuation and enhancement of the organ or an alteration of its typically triangular shape in cases of thymic neoplasia (Fig. 9-17), CT often does not definitively assess the thymus for tumor. [93]

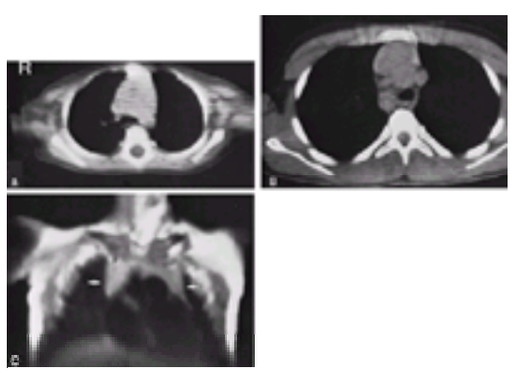

**FIGURE 9-17.** Thymus gland. **A:** Computed tomography scan through the thymus shows a normal triangular gland between the heart and sternum. **B:** A rounded, tumor-infiltrated thymus is seen in a 14-year-old girl with Hodgkin's disease. **C:** A T1-weighted coronal scan of the chest of an 8-year-old boy demonstrates both lobes of the thymus (*arrows*) to good advantage.

MR studies of the thymus may be able to differentiate thymic abnormalities from normal thymus in many instances. [94] On MR, lymphoma of the thymus can be seen as asymmetric or diffuse enlargement of the gland. There may be marked inhomogeneity of the thymus, with intermediate signal intensity on the T1-weighted image and bright signal intensity on the T2-weighted image, which may be caused by underlying necrosis or cystic degeneration ( Fig. 9-18). The normal thymus has a homogeneous intermediate signal intensity on T1-weighted images, with a homogenous mildly increased signal intensity (similar to that of subcutaneous fat) on T2-weighted images. This pattern, combined with the size and contour of the gland, often allows differentiation of normal from abnormal thymus on MR scans. Gallium and PET scanning can also be helpful in identifying malignant thymic disease and specifically in differentiating thymic rebound from recurrent tumor.

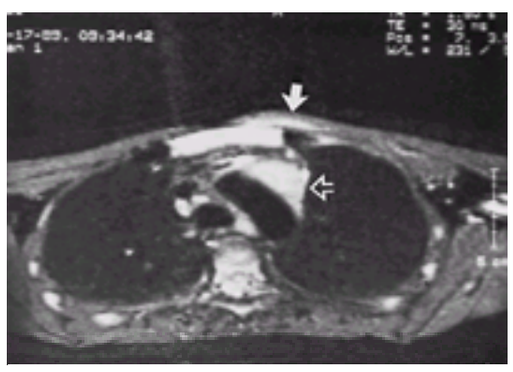

**FIGURE 9-18.** Axial short T1 inversion recovery image of a patient with lymphoma shows chest wall involvement (*closed arrow*) and paraaortic involvement (*open*

*arrow*). The sternal marrow signal is also very high. (Courtesy of Dr. Colleen Bergin, Stanford, CA.)

Pleural involvement by malignant tumors is most typically from metastatic disease and is discussed in the portion of this chapter devoted to tumor staging. Pleural involvement by lymphoma may be evaluated by MR imaging or CT.

Chest wall tumors may arise from bone or soft tissues. The most common malignant soft tissue lesions are rhabdomyosarcoma, extraosseous Ewing's sarcoma, and primitive neuroectodermal tumor or Askin's tumor. Although plain films may demonstrate a nonspecific mass, CT allows evaluation of the extent of tumor, is more sensitive for bony erosion, and may accurately determine the site of origin of tumors arising in unusual places such as the diaphragm. MR can also be used to evaluate chest wall tumors and is superior to CT in evaluating individual components of the chest wall, including muscle, fat, lymph nodes, vessels, and nerves ( Fig. 9-1). By using a surface coil, the marrow and subtle cortical detail of the ribs can usually be evaluated. However, if the cortex is not adequately seen on the MR examination, CT can be used as an ancillary examination specifically to evaluate the cortical bone. Primary bone tumors, especially Ewing's sarcoma and some osteogenic sarcomas, may arise from the ribs, thoracic vertebrae, or scapulae and manifest as chest masses. CT and MR evaluation may give specific diagnostic information as well as general information concerning the extent of tumor and the presence of mediastinal disease; CT is superior in the evaluation of pulmonary metastases.

***Abdominal Masses***

The plethora of diagnostic imaging examinations available for evaluation of abdominal masses in infants and children requires a logical and analytic approach to avoid unnecessary expense, radiation, and potential morbidity. In neonates, more than 85% of abdominal masses are nonmalignant. [95] A combination of plain films and abdominal ultrasound usually identifies the organ of origin and, if the mass is not solid, suggests its benignancy. Renal abnormalities account for more than one-half of the palpable abdominal masses in the neonatal period, with hydronephrosis and renal cystic dysplasia accounting for the majority of these. Ultrasound can usually determine a specific diagnosis in these cases.

The next most common cause of abdominal masses in the neonatal period, cephalad extension of pelvic, usually ovarian, lesions in infant girls, is also readily amenable to ultrasound diagnosis. Ultrasound has also been particularly useful in differentiating neonatal adrenal hemorrhage from neonatal adrenal neuroblastoma. Lesions that develop central hypodensity and, most important, decrease in size over time are likely evolving hemorrhages whereas those that increase in size are presumed to be neuroblastomas until proven otherwise.[96] If a combination of plain film radiography and ultrasound identifies a solid tumor arising from the retroperitoneum or within the liver, the probability of malignancy increases, and further imaging evaluation by either CT or MR is usually necessary.

Nonmalignant GI abnormalities presenting as palpable abdominal masses in neonates, such as large duplication or choledochal cysts or a distended stomach or bowel segment, are commonly accompanied by bowel obstruction evident on conventional abdominal radiographs and can be further evaluated by the appropriate fluoroscopically monitored GI contrast examinations. Sonography and scintigraphy can also be helpful.

In infants beyond the neonatal period and in children, the likelihood that an intraabdominal mass reflects malignant rather than benign disease increases. Nevertheless, malignancy still accounts for less than one-half of the palpable abdominal masses in infants and children. Plain film radiography and ultrasound again should be the primary screening imaging modalities, with nuclear scintigraphy, CT, and MR imaging used for further evaluation as indicated. As in neonates, cystic or fluid-filled masses typically are benign; solid masses always have to be considered malignant until proved otherwise.

***Retroperitoneal Masses***

Most solid extracranial tumors in infants and children arise from the kidney or adrenal gland. Mesoblastic nephroma, a generally benign tumor, accounts for the majority of solid renal masses in children younger than 1 year of age, but the tumor is indistinguishable from Wilms' tumor on imaging examinations and requires biopsy for definitive diagnosis. For children older than the age of 1, nearly all solid tumors arising in the kidney are Wilms' tumors. The combination of ultrasound and CT typically provides sufficient information about the size and location of the mass, vascular invasion or compromise, and lymph node and hepatic metastases for surgical planning (Fig. 9-9). Some surgeons, however, have questioned the need for routine CT examinations of Wilms' tumor patients, and the accuracy of radiographic staging has been controversial. [97],[98] and [99]

Most nonrenal retroperitoneal solid tumors are neuroblastomas. [100] No current imaging modality can reliably differentiate neuroblastoma from ganglioneuroblastoma and ganglioneuroma. Although some teratomas are indistinguishable from neuroblastomas on imaging studies, most of them have sufficient evidence of multitissue origin on ultrasound and CT to enable a correct diagnosis to be suggested. Because most teratomas contain fat, MR is an excellent tool for making a precise diagnosis.

Wilms' tumors and neuroblastomas may be so large as to make accurate assessment of the organ of origin difficult by ultrasound, [101] but CT with intravenous contrast enhancement is usually quite accurate for this purpose. Several studies have suggested that MR may be superior to ultrasonography and CT in the diagnosis and staging of Wilms' tumor.[102] However, these studies were performed with small numbers of patients, and MR is performed routinely in such patients in only a limited number of institutions. The primary potential advantage of MR over CT is the ability of MR imaging to evaluate the renal vein, inferior vena cava, and other surrounding vascular structures without the need for intravenous contrast. A second advantage is the ability to directly visualize the tumor in the coronal and sagittal planes. This allows a more accurate evaluation of the liver and surrounding structures and can aid in differentiating hepatic, renal, and adrenal tumors ( Fig. 9-19). On MR, most Wilms' tumors are heterogeneous, with primarily low signal intensity on T1-weighted images and heterogeneous areas of intermediate and increased signal intensity on T2-weighted images. Much of the heterogeneity appreciated on the T2-weighted images is secondary to regions of necrosis and hemorrhage within the tumor.

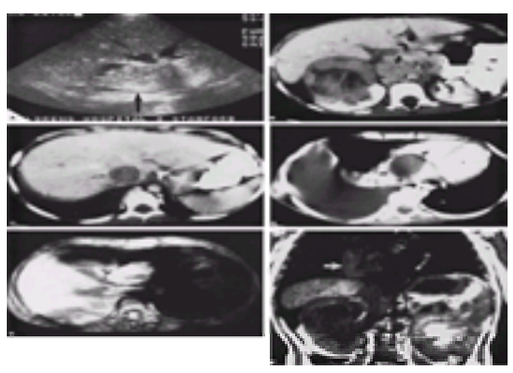

**FIGURE 9-19.** Wilms' tumor. **A:** Ultrasound demonstrates a large mass (*arrow*) within the inferior vena cava (IVC), causing obstruction of hepatic veins. **B:** Computed tomography (CT) demonstrates a large, right renal mass with massive retroperitoneal adenopathy (*solid arrows*) surrounding an opacified aorta (*open arrow*). **C:** CT at a higher level demonstrates tumor thrombus in the IVC (*arrow*). **D:** CT at a still higher level demonstrates tumor thrombus in right atrium (*arrows*) and right pleural effusion. **E:** An axial magnetic resonance (MR) scan demonstrates tumor thrombus in the right atrium and a right pleural effusion (same level as **D**). **F:** Coronal MR scan demonstrates tumor from the right kidney extending into the IVC and right atrium (*arrow*).

Other retroperitoneal masses can be identified, but specific diagnoses may be difficult to make. Rhabdomyosarcoma, fibromatosis, fibrosarcoma, and other tumors do not have specific imaging characteristics, although precise evaluation of their location may suggest the correct diagnosis. Patients with lymphoreticular malignancies commonly have nonspecific retroperitoneal lymphadenopathy, but other manifestations of the disease usually lead to the proper diagnosis.

### *Intraperitoneal Masses*

Most intraperitoneal masses in infants and young children are either related to abnormal bowel and typically diagnosable by a combination of plain film and barium studies, or are cystic, likely benign, and diagnosable by ultrasound. Hepatic tumors and lymphoma account for the majority of intraperitoneal abdominal masses that are identified as solid by ultrasound. Lymphomas can appear relatively hypoechoic, mimicking fluid-filled masses, but careful ultrasonic examination usually can correctly determine the solid nature of the mass and should be followed by abdominal CT scanning for staging purposes.

MR cine angiography is increasingly used in the setting of hepatic neoplastic disease to evaluate the hepatic vasculature and intravascular extension of tumor ( Fig. 9-20). Several studies have compared MR with CT in the evaluation of hepatocellular carcinoma. [103,104] These studies showed that MR is superior to CT in the detection of a pseudocapsule, the evaluation of vascular involvement, and the identification of internal tumor architecture. MR also was shown to be superior to CT in identifying tumor recurrence. On MR, liver tumors usually demonstrate intermediate signal intensity on T1-weighted images and increased signal intensity on T2-weighted images. The internal architecture is variable and ranges from homogeneous to markedly heterogeneous. [105] Calcification is usually not well seen on MR examination. MR angiography commonly performed with intravenous bolus injection of gadolinium contrast agents permits imaging of the hepatic arterial supply, portal vein, and hepatic veins. The role of contrast-enhanced MR imaging of the substance of the tumor itself is not yet established. Preliminary results indicate that DEMRI may differentiate hypovascular from hypervascular lesions and be prognostically useful. [106]

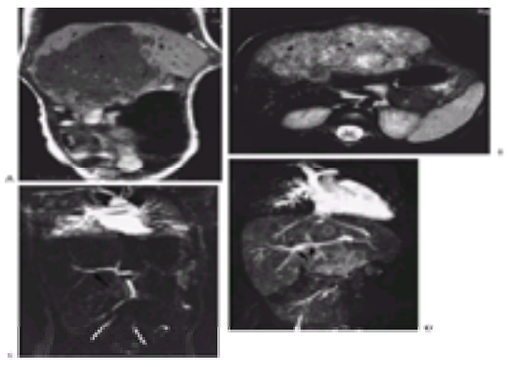

**FIGURE 9-20.** Hepatoblastoma. Coronal T1-weighted **(A)** and axial T2-weighted **(B)** magnetic resonance (MR) imaging shows a large intrahepatic tumor (*stars*). **C:** MR arteriogram demonstrates the hepatic artery (*arrow*). **D:** MR venogram demonstrates the central left and right portal veins (*closed arrows*) and laterally displaced left hepatic vein (*open arrow*). (Courtesy of Dr. Taylor Chung, Department of Diagnostic Imaging, Texas Children's Hospital, Houston, TX.)

### *Pelvic Tumors*

Rhabdomyosarcoma is the most common pelvic tumor in boys; ovarian tumors and rhabdomyosarcomas must be considered in girls. Although cystography and excretory urography have been widely used in the past for evaluation of rhabdomyosarcomas arising in or near the urinary bladder, MR and contrast-enhanced CT scanning allow evaluation of tumor masses in the intravesical and extravesical areas and supply information both about the primary tumor and about any intraabdominal metastases that might be present.

MR has some advantages over CT in the evaluation of pediatric pelvic tumors, and in some institutions MR is the examination of choice. The greatest advantage of MR over CT is its ability to directly image in the coronal, sagittal, and axial planes. This is particularly useful in delineating rhabdomyosarcoma, for which invasion of the posterior aspect of the bladder wall and the anterior aspect of the rectum is an important finding. The ability to evaluate the bladder and to assess the upper urinary tracts for obstruction without the use of intravenous contrast is another advantage. Both MR and CT can identify enlarged pelvic lymph nodes, an important capability critical to staging of intrapelvic neoplasms. As stated, the disadvantages of MR include its high cost, its limited availability, its susceptibility to motion, and the absence of a widely utilized bowel contrast agent.

Most ovarian tumors are cystic and benign and are identified as such by ultrasound. Teratomas may appear echogenic or echolucent but frequently have mixed characteristics and calcifications that may suggest the correct diagnosis. Although malignant ovarian tumors are uncommon in childhood, they appear solid on ultrasonographic examination or have mixed solid and cystic areas ( Fig. 9-21). If necessary, they can be more thoroughly evaluated by means of pelvic and abdominal MR or CT scanning.

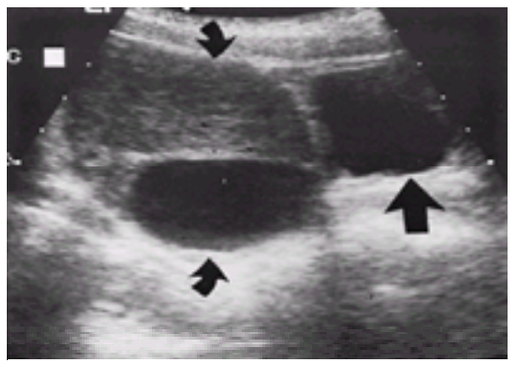

**FIGURE 9-21.** Ovarian dysgerminoma. Pelvic sonogram discloses a mixed solid and cystic tumor (*curved arrows*) superior to the bladder (*straight arrow*).

### *Miscellaneous Abdominal Masses*

Malignant disease involving the spleen characteristically is infiltrative in nature and causes splenomegaly rather than a discrete splenic mass. This is especially true of leukemia. Lymphomatous involvement of the spleen can be diffusely infiltrative in nature, can result in discrete tumor foci, or can cause a combination of both of these types of disease. Lymphoreticular malignancies isolated to the spleen are extraordinary, so the diagnosis is usually apparent on the basis of extrasplenic lesions. Splenic cysts are typically hypoechoic, and careful ultrasonography usually obviates the need for additional studies. Discrete hepatic and splenic metastases from solid tumors and lymphomas can be identified by CT if they measure at least 3 mm in diameter, and many smaller lesions are also detectable by this modality. MR has a similar sensitivity to CT in evaluating the liver and spleen, and ultrasound is only slightly less sensitive. Of note, because most patients with primary abdominal tumors have CT or MR scans and many have ultrasound studies as well, these examinations of the primary tumors are typically sufficient to evaluate for any accompanying hepatic and splenic metastases that might be present.

Although tumor-laden lymph nodes in the porta hepatis may cause biliary obstruction, most biliary tract pathology in children is cystic and benign and easily evaluated by ultrasound and nuclear medicine studies. Most pancreatic lesions in children are also cystic and benign. They are commonly evaluated by ultrasound but can also be studied by CT and MR. MR cholangiopancreatography has proven to be effective even in very young patients and in many institutions has largely replaced endoscopic retrograde cholangiopancreatography in the evaluation of the pancreaticobiliary tree. Rarely, solid masses in the pancreas can be found, and these typically represent metastatic disease, lymphoma, pancreaticoblastoma, or rare endocrine tumors. Unless the patient already has a known primary, the discovery of a solid pancreatic abnormality on ultrasound should prompt CT or MR scanning for further evaluation.

### *Extremities*

Osteogenic sarcoma and Ewing's sarcoma are the most common primary bone tumors of childhood, but when bone lysis is encountered, all members of the group of conditions known as the “small round cell tumors of childhood” must be considered in the differential diagnosis (see Chapter 8). These tumors include metastases, especially from neuroblastoma, rhabdomyosarcoma, Wilms' tumor, leukemia, and lymphoma, as well as Langerhans' cell histocytosis and osteomyelitis. An algorithmic approach based on numerous criteria may narrow the differential diagnosis, [107] but an exact diagnosis is not always possible. Most osteogenic sarcomas make tumoral bone and can be diagnosed on plain film radiography ( Fig. 9-5). However, not all osteogenic sarcomas manifest with obvious malignant bone formation, and it not always possible to make a definitive diagnosis, especially in the earliest stages of the disease (see Chapter 35). Patients presenting with pain and unusual fractures should have osteogenic sarcoma and other malignancies considered ( Fig. 9-22). Small destructive lesions of bone associated with soft tissue masses should also raise the possibility of early osteogenic sarcoma. When a combination of clinical information and radiographic appearance makes a primary bone tumor likely, an MR scan should be obtained, which typically very accurately demonstrates the extent of intramedullary disease and of associated extraosseous malignancy and edema and can do so in multiple planes.[108]

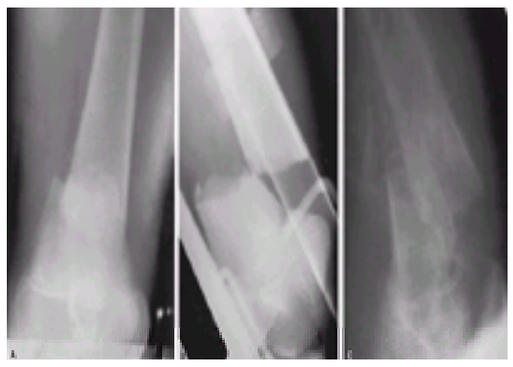

**FIGURE 9-22.** Osteosarcoma in a 13-year-old boy. Anteroposterior **(A)** and lateral **(B)** views of the distal femur show a transverse fracture after trauma. Transverse fractures are unusual after infancy and frequently indicate an underlying pathologic process. **C:** Follow-up examination 3 weeks later demonstrates irregular edges of the fracture and tumoral new bone, suggesting a malignant process.

## Staging

Imaging procedures are invaluable in the staging of most solid tumors and may come to play a role in the evaluation of acute leukemia ( Table 9-4). Although the staging procedures used depend on tumor type and location, the underlying principle is evaluation of local and distant extent of tumor. Local extent is usually evaluated by the primary diagnostic modalities discussed previously, and this section deals with the evaluation of common areas of metastatic disease.

| |
|---|
| Central nervous system |
| MR |
| Lungs and mediastinum |
| CT without contrast for lungs only and with and without contrast for mediastinum |
| Liver |
| Enhanced CT (or MR) for primary evaluation; ultrasound for ease of follow-up |
| Retroperitoneal and intraperitoneal lymph nodes |
| CT |
| Bones |
| Radioisotope bone scan with plain films of areas positive on bone scan |
| Exceptions: Langerhans' cell histiocytosis and neuroblastoma in which a combination of both plain film skeletal surveys and total body bone scintigrams may be appropriate. |

CT, computed tomography; MR, magnetic resonance.

**TABLE 9-4. RECOMMENDED IMAGING EVALUATION FOR METASTATIC DISEASE**

### *Central Nervous System Metastases*

Metastatic disease to the brain, the spinal cord, and the meninges can be evaluated by CT but is more typically studied by MR. Of note, a major advantage of MR for detection of metastatic lesions within the spinal canal is obviation of the need for intrathecal contrast material typically used in conjunction with CT scanning. Although there is a lack of signal from cortical bone on MR studies, metastatic involvement of adjacent cranial and vertebral structures can usually be identified on MR by changes in the marrow signal.

### *Pulmonary Metastases*

Although metastases to the lung can be seen on chest radiographs, CT is a far more sensitive imaging modality for this purpose. CT frequently identifies metastases 2 to 3 mm in diameter, which may not be seen on plain chest films (Fig. 9-23). CT also can identify metastatic lesions at the periphery of the lungs, a problem area for plain films. Spiral CT has been especially valuable for cooperative patients because of the lack of “skip areas” found with conventional CT. The major disadvantage of CT has been its inability to differentiate metastases from benign lesions, especially granulomas, which may appear in the lungs. However, children are far less likely to have granulomas than adults, and nodular lesions in the lungs of children with known malignant solid tumors, though they are often benign, should be considered metastases until proved otherwise. Although its ability to assess the lung parenchyma is superb, CT is somewhat less successful in differentiating subtle hilar masses and nodes from normal vascular structures, and when such lesions are potentially present, MR imaging can be helpful.

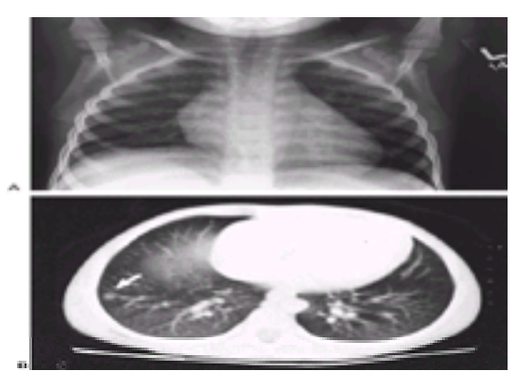

**FIGURE 9-23.** Wilms' tumor. **A:** Anteroposterior chest radiograph demonstrates a clear lung. **B:** Computed tomography of the lungs demonstrates a pulmonary nodule (*arrow*).

Patients with pleural metastases frequently have large effusions, which makes evaluation of the pleural surfaces and the lungs difficult by conventional chest radiography. CT has the advantage of often allowing visualization of underlying thoracic structures even in the presence of pleural effusion ( Fig. 9-24) and can facilitate percutaneous pleural biopsy when necessary. The imaging approach to mediastinal metastatic disease is the same as that for the primary intramediastinal tumors discussed previously.

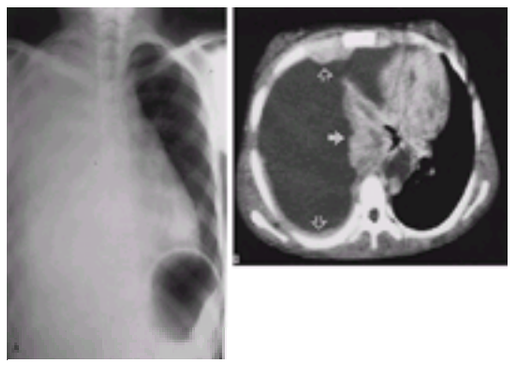

**FIGURE 9-24.** Non-Hodgkin's lymphoma in a 13-year-old boy. **A:** A chest radiograph shows a large right pleural effusion of unknown cause. **B:** A computed tomography scan demonstrates a collapsed lung (*closed arrow*) and pleural plaques of tumor (*open arrows*), one of which was biopsied for diagnosis.

### *Intraabdominal Metastases*

The liver represents the most common site of metastatic disease within the abdomen, and the organ is assessed reasonably well by ultrasound and even better by CT and MR. In many but not all of the studies performed comparing CT to MR in the detection of metastatic disease in adults, MR was found to be more sensitive than CT, even when the CT imaging was performed during the intraarterial infusion of iodinated contrast agent, so-called “CT angiography.” [109,110] Nonetheless, in most instances CT is entirely adequate to investigate possible metastatic disease. As most liver metastases arise from solid intraabdominal tumors, the CT and MR examinations invariably performed to assess the primary lesions suffices to evaluate for possible accompanying hepatic metastatic disease. These studies also evaluate the spleen, adrenal glands, pancreas, and kidneys for possible metastases. Although less sensitive than CT or MR, ultrasound can be a valuable adjunct in the evaluation of metastatic disease, including liver metastases, and is especially useful for short-term interval follow-up studies for which the time, the expense, and, in some cases, the necessity for sedation or anesthesia make CT or MR less feasible.

The retroperitoneal lymph nodes (Table 9-2) can be evaluated for metastases from nonlymphomatous tumors with 92% accuracy by lymphangiography and by CT scans with 84% accuracy.[111] However, CT scanning is not able to differentiate lymph nodes enlarged because of benign disease from those enlarged because of tumor infiltration. CT also suffers from an inability to identify normal-sized lymph nodes that may be infiltrated by tumor. Although reactive hyperplasia causes mild enlargement of lymph nodes, moderate to massive enlargement usually suggests infiltration by tumor. In such cases, CT may give sufficient diagnostic information. If more detailed information is required, lymphangiography can be performed. MR also can identify abnormally enlarged retroperitoneal lymph nodes, but its ability to identify enlarged lymph nodes is not greater than that of CT scanning and it too cannot diagnose tumor in normal-sized nodes nor distinguish between tumor and benign hyperplasia as the cause of enlargement of any particular node.

### *Skeletal Metastases*

Skeletal metastases may be found in patients with most solid tumors, and bony infiltration may be seen in patients with lymphoreticular malignancies, including Langerhans' cell histiocytosis. Radioisotope bone scans are generally more sensitive than plain film radiographs for the detection of metastatic disease and constitute the usual means for the initial diagnosis and the follow-up of bony metastases. [112] An exception to this rule is Langerhans' cell histiocytosis and neuroblastoma in which a combination of both plain film skeletal series and total body bone scintigraphy can be helpful to optimize lesion detection. [113,114 and 115]

In general, CT and MR imaging should be reserved for the evaluation of bony metastases when the patient presents with clinical symptoms (e.g., bone pain) but has negative plain film and scintigraphic results. MR is preferable to CT when evaluating these metastatic lesions because of its increased sensitivity to marrow disease and superior demonstration of associated extraosseous masses and edema, information that is critical if the lesion is to the irradiated or surgically resected. CT can demonstrate the cortical bone better than MR but is seldom necessary. In addition to assessing focal bony metastases, MR is also useful when evaluating for diffuse bone marrow infiltration by tumor, a condition that typically presents with abnormally decreased T1-weighted marrow signal ( Fig. 9-25).

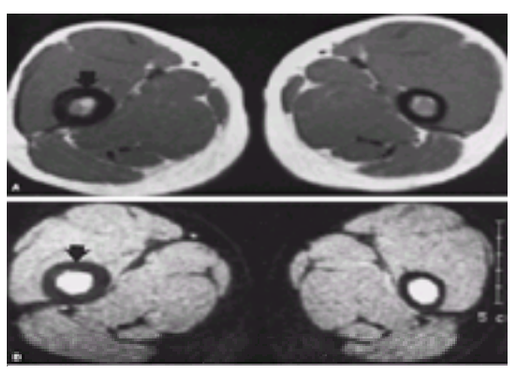

**FIGURE 9-25.** Axial **(A)** T1-weighted and short t inversion recovery (STIR) **(B)** magnetic resonance images through the femora of a child with diffuse bilateral femoral metastases from neuroblastoma. On the T1-weighted image, the marrow signal intensity is intermediate, and it is unclear on this image whether the marrow is abnormal. The cortex surrounding the femur, however, is abnormally thickened (*arrow*). On the STIR image, the bright signal intensity of the marrow belies the diffuse metastatic disease. Thickening of the right femoral cortex is again evident along with some minimal abnormally increased intracortical signal ( *arrow*).

## Guide to Therapy

Accurate assessment of tumor volume and local extent is particularly important when surgery, radiation therapy, or both, are used. Generally, the imaging studies recommended for primary tumors in previous sections of this chapter and in the disease-specific chapters of this book give adequate information for the surgeon or radiation therapist. Diagnostic arteriography is rarely indicated but, as noted, is occasionally performed for liver and musculoskeletal tumors, particularly before resective surgery.

## Evaluation of Therapy

Therapeutic responses can be evaluated with the same imaging modalities used in evaluation of the primary tumor. In most instances, CT or MR is the modality of choice. However, if ultrasound has been satisfactory in delineating the primary tumor, this method should be used for following the response to therapy because it is less costly and requires no sedation or anesthesia. Metastatic lesions are also best followed using the same modality that demonstrated them at the time of presentation and diagnosis.

There is at present considerable controversy regarding the most appropriate parameter to gauge changes in tumor size. Some investigators have advocated the reporting of one, two, or three maximal lengths in orthogonal planes. Others have suggested that the multiple of two maximal lengths in orthogonal planes be used and others that estimates of tumor volume be used.[116,117,118,119 and 120]

Physiologic assessments of therapeutic response by PET, MRS, and quantitative DEMRI may also play a significant role in tumor management in the future.

**Detection of Recurrences**

Recurrent disease can be defined as reappearance of tumor in its original location or as metastases in distant sites. The primary site is usually best followed with the imaging modality used for the initial tumor. Adequate evaluation for metastatic disease requires knowledge of the natural history of the tumor. Chest films, chest CT, craniospinal and extremity MR, bone scans, and abdominopelvic ultrasound, CT, and MR scans are all reasonably used depending on the site of the original tumor.

The vexing problem, which has yet to be resolved, is how often follow-up studies should be obtained. A study of recurrent Hodgkin's disease in children showed that 56% of patients who relapsed developed their recurrences within 2 years and 71% within 3 years of presentation. [121] However, an overall relapse rate of 48% in the years before 1970 has dropped to less than 10% since 1970. This reflects inadequate staging in the early years and improved therapy and better staging in recent years. Close-interval follow-up imaging procedures can detect recurrences, but the population at risk is quite small.

Studies of the value of CT in the follow-up of abdominal neuroblastoma [122] demonstrated patients with stage III or IV disease to be at high risk for recurrence during the first 18 months after presentation. These researchers recommend close-interval follow-ups, with CT scans every 12 weeks during this high-risk period. Patients with stage I and II disease were at low risk of recurrence, and a single CT scan 12 weeks after surgery was recommended. A more recently completed study, however, suggested that in patients with advanced-stage neuroblastoma history, physical examination and selective laboratory evaluation were more sensitive and cost-effective than radiographic imaging in detecting progressive and recurrent disease. [123]

Statistical analyses of recurrent disease are complex but feasible, and several have been performed and published. [121,124,125] One of these studies suggests that follow-up chest radiographs are obtained too frequently for pediatric patients with Hodgkin's disease, [121] and the authors suggested an optimal frequency of every 3 months after the onset of remission for 18 months and then every 6 months for another 3 years for patients with this disease. A study of patients with Ewing's sarcoma suggested that these patients are at risk for bone metastases for 3 years if they had truncal primaries and for 4 years if they had extremity primaries. [125] However, therapeutic results have improved dramatically since this study was done, modifying the natural history of treated Ewing's tumor. A study of bone metastases in patients with osteosarcoma demonstrated a linearly increasing risk for 5 to 29 months after diagnosis. [126]

Although these statistical analyses represent the best way to evaluate the required frequency of follow-up studies, the changing natural history of most treated children's tumors makes accurate assessment of optimal timing of follow-up studies difficult. Table 9-5 suggests a plan of follow-up that fits the current situation.

**TABLE 9-5. RECOMMENDED FOLLOW-UP IMAGING PROCEDURES**

**Complications of Antitumor Therapy**

All three primary modalities used in antitumor therapy (surgery, radiation therapy, and chemotherapy) may lead to complications that can be diagnosed by imaging procedures. Most complications of surgery, including infection, bleeding, scarring, and adhesion formation, are not unique to cancer patients and are not dealt with further in this chapter. Complications of radiation therapy and chemotherapy can be described as direct effects, such as radio-osteonecrosis and cardiomyopathy, or secondary effects, such as steroid-induced ulcers or infections consequent to immunosuppression.

From the viewpoint of the imaging specialist, much attention historically has been paid to the effects of radiation therapy. Children are more likely than adults to develop complications of radiation therapy because growing bone is more sensitive to irradiation than the more stable adult bone. Interference with normal bone growth may lead to shortening of a radiated bone or overall diminution in height from spinal irradiation. [127] In long bones, characteristic deformities similar to those seen in patients with trauma and rickets have been described. [128] Loss of spinal height is best determined clinically, but radiographs typically show failure of maturation of the vertebral bodies in irradiated fields. [129] Spinal irradiation may also lead to scoliosis with secondary loss of height, but the average angle of radiation-induced scoliosis is less than 10 degrees, infrequently leading to clinically apparent abnormalities. Other areas in which the impact of radiation on growth is seen include hypoplasia of the clavicles and the iliac bones when they are included in radiation fields.

Depending on dose-time relations, the impact of radiation on mineralization ranges from mild osteoporosis to frank radio-osteonecrosis. Because profound bone necrosis has a radiographic pattern simulating small-cell infiltration, the differentiation between radiation effect and recurrent or metastatic disease can be difficult on both conventional radiographs and CT. Radio-osteonecrosis does not cause rupture of the bony cortex and tends to be stable over successive examinations whereas infiltrative bone disease usually breaks through the cortex into the subperiosteal region and shows progression over fairly short intervals of time. MR can detect radionecrosis and can often distinguish it from tumor. On the MR scan, there is increased signal intensity for muscle, bone, and bone marrow, and deep fascial planes, which corresponds to the radiation port. Fluid also may be seen in the region. These changes represent the diffuse inflammation and edema associated with radiation necrosis. In the bone marrow, these changes appear feathery and ill defined and usually can be differentiated from focal metastatic lesions.

Radiation-induced tumors of bone are most commonly benign, with osteochondroma being the most frequently observed lesion. [130] These osteochondromas typically arise from growth plates in non-weightbearing bones. However, they can arise in virtually any bone in which the growth plate is irradiated. They have been described in patients receiving doses as small as 12 Gy. Many of these tumors have been removed surgically and have been found to be benign. If not removed, they should be followed carefully with periodic examination, because any osteochondroma has the potential to undergo malignant degeneration.

Radiation-induced malignant tumors are a well-known phenomenon. Osteosarcoma is the most common, although chondrosarcomas and fibrosarcomas are also seen. The latent period for sarcoma development is 4.5 to 27.0 years, with a median time of 11 years and a mean time of 12 years. [131]

Cartilage necrosis may occur when joints are included in the radiation field, and resultant calcification of articular cartilage may be identified on plain films. Similarly, conventional radiographs can demonstrate ischemic necrosis of the humeral heads and femoral heads, [132] although scintigraphy and MR are more sensitive in detecting such necrosis.[133,134] Muscle atrophy may be suggested on plain films but is easily evaluated on MR, manifesting as diminished muscle bulk and increased intramuscular signal on T1-weighted imaging reflecting fatty replacement of muscle tissue.

Radiation can also have significant deleterious effects on nonmusculoskeletal structures. Irradiation of the brain, usually in conjunction with intrathecal chemotherapy,

may lead to leukomalacia and, occasionally, calcification that can be identified on CT [135] and MR scans. Frank brain necrosis may occur with high doses.

The thyroid gland is particularly susceptible to radiation damage, and thyroid cancers may be identified by nuclear medicine studies, ultrasound, CT, or MR. Acute radiation reaction in the lungs is well demonstrated on chest films, appearing as air space and interstitial infiltrates within a well-defined anatomic area corresponding to the radiation field. Acute changes usually appear near the end of therapy or within a month of the completion of therapy and may last 2 to 9 months. If the dose is high enough, chronic interstitial fibrosis may develop that is well circumscribed within the radiation port. CT is more accurate than conventional chest radiographs in detecting the earliest changes of radiation fibrosis. [136] High-resolution CT is particularly sensitive for identifying even minimal pulmonary fibrosis. [33]

The GI mucosa is highly susceptible to radiation damage. Barium studies are the best way to evaluate esophagitis and enterocolitis, because they show mucosal changes and bowel tethering due to mesenteric disease most definitively. CT and ultrasound can, however, also be of help in demonstrating findings of radiation-induced GI tract disease. Neutropenic enteritis, which can occur with total body irradiation for bone marrow ablation, with aggressive chemotherapy, or with a combination of these two therapies, can be assessed by ultrasound or CT. [137,138]

The liver and kidneys can also be damaged by irradiation. Radiation hepatitis has been well defined on radioisotope liver scans as areas of diminished radionuclide uptake corresponding to the radiation portals. Isotope liver scans may be abnormal in both the acute and chronic phases of radiation hepatitis. Because such hepatitis may mimic metastatic disease on scintigraphy, further investigation of a suspicious finding on an isotope liver scan by ultrasound, MR, or CT scanning may be helpful. Irradiation of the immature kidney may result in a failure of normal growth. [139] Serial ultrasound examinations are excellent for determining and comparing renal size and, in such cases, can demonstrate a lack of growth of the irradiated kidney over a period of several years.

The adverse effects of chemotherapy can also be evaluated with imaging studies, depending on the organ involved. Leukomalacia after intrathecal chemotherapy, usually in association with radiotherapy, has been defined on CT and MR studies. Pulmonary toxicities that can be demonstrated by plain films and high-resolution CT include reversible processes such as bronchiolitis obliterans with organizing pneumonia ( Fig. 9-26) and irreversible fibrosis. [140] Radiographic abnormalities, however, often occur relatively late in the course of lung toxicities, and pulmonary function tests can be more sensitive than imaging studies for detecting early changes. Cardiomyopathy secondary to anthracycline therapy causes cardiomegaly and pulmonary edema in later stages. However, early changes can be evaluated by calculating left ventricular ejection fractions by echocardiography, radionuclide techniques, or MR imaging.

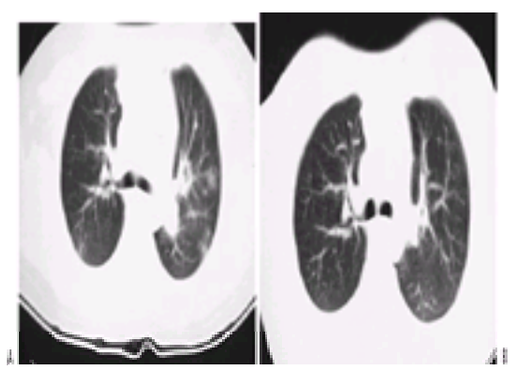

**FIGURE 9-26.** Bronchiolitis obliterans with organizing pneumonia in a patient who had undergone bone marrow transplantation for acute lymphocytic leukemia. **A:** An initial chest computed tomography scan shows scattered patchy pulmonary opacities. **B:** A follow-up study performed 9 months later after treatment with steroids shows resolution of the previously depicted pulmonary process.

The secondary effects of chemotherapy agents can also be diagnosed by imaging. The most common are infections secondary to immunosuppression. These are dealt with in Chapter 41. The other common group of secondary effects is related to steroids and include such radiographically demonstrable abnormalities as pseudotumor cerebri, retarded skeletal maturation, ischemic necrosis of the femoral heads, diffuse osteoporosis with compression fractures of vertebral bodies, abnormal fat deposition, and gastric and duodenal ulcers. The appropriate imaging study to identify the abnormality depends on the anatomic region involved.

## FUTURE CONSIDERATIONS

In the first edition of this book, we identified several areas for potential advancement in digital radiography, MR, CT, ultrasound, and nuclear medicine, including PET scanning. Great progress has been made in the technology for digital image acquisition and for the electronic archival, disbursal, and display of diagnostic images, technologies that are now available at many institutions throughout the world. The utility of MR has also continued to advance rapidly with the introduction of faster sequences and innovations that have increased the signal-to-noise ratio, improved spatial resolution, and decreased susceptibility of the modality to motion artifact. MR diffusion imaging has been introduced and MR vascular imaging refined. CT imaging speed has continued to increase as has the availability of advanced computer hardware and software necessary for the manipulation of CT data to facilitate multiplanar reconstruction, three-dimensional rendering, and CT angiography.

Advances in other areas have been more limited. The potential value of *in vivo* MRS continues to be exciting but speculative and controversial. Several major centers are investing heavily in MRS equipment and research, although at present the technique has yet to significantly impact the clinical care of children with neoplasia. Doppler ultrasound evaluation of blood flow within tumors has not proven helpful in distinguishing malignant from benign masses, or in facilitating prognostication or assessing tumor response in known malignancies. The introduction of innovative therapeutic radiopharmaceuticals, such as radio-labeled monoclonal antibodies, has not yet impacted the treatment of pediatric tumors, nor has PET scanning, to this point in time, been embraced as a critical modality for the assessment of childhood malignancies.

The medical economic climate has had a profound effect on medical imaging and clinical medicine. Managed care and per diem payments by third-party payers have changed radiology from a profit center to a cost center in many instances. Radiologists must carry out rigorous studies of efficacy and outcome to determine the best imaging approach to diagnostic problems and, more important, the proper follow-up of treated patients. The large cooperative study groups such as the Children's Oncology Group, provide an opportunity for us to conduct controlled trials with proper protocols, and radiologists are more involved in these studies than at any previous time.

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# 10

# GENERAL PRINCIPLES OF CHEMOTHERAPY

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## INTRODUCTION

Since the introduction of chemotherapy for the treatment of childhood leukemia in the 1940s, [1] the prognosis of childhood cancer has improved dramatically ( Fig. 10-1). The 5-year survival rate for this group of diseases, many of which were uniformly fatal in the prechemotherapy era, was 75% for all forms of childhood cancer diagnosed between 1989 and 1995. [2] This striking improvement in survival is a direct result of the incorporation of anticancer drugs into treatment regimens that previously relied only on surgery or radiotherapy for the primary tumor. [3] The multimodality approach, which integrates surgery and radiotherapy to control local disease with chemotherapy to eradicate systemic (metastatic) disease, has become the standard approach to treating most childhood cancers.

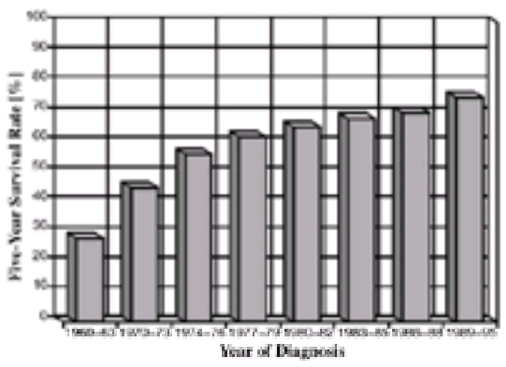



**FIGURE 10-1.** Five-year survival rate for all childhood cancers diagnosed between 1960 and 1995. (Data from Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer statistics, 2000. CA Cancer J Clin 2000;50:7.)

## PRINCIPLES OF CANCER CHEMOTHERAPY

The ultimate goal of the multimodality treatment approach, in which anticancer drugs play a critical role, is to cure the patient of his or her cancer. The feasibility of achieving cures by the addition of anticancer drugs to surgery or radiation was first demonstrated in chemosensitive childhood cancers, such as Wilms' tumor. [4] However, curing the underlying disease is not the goal of most pharmacologic interventions. With the exception of antimicrobial and anticancer chemotherapy, the common classes of drugs (e.g., antihypertensives) are administered with the intent of controlling the disease or the symptoms caused by disease, rather than curing the underlying disease. The model for curing cancer is based on the successful model of curing bacterial infections. This strategy attempts to exploit differences between cancer and normal host cells and eradicate or kill all cancer cells in the body. This “killing paradigm” [5] has had a profound impact on the approach to anticancer drug discovery, drug development, and the design of treatment regimens that incorporate anticancer drugs.

The predominant strategy for anticancer drug discovery has been high-throughput screening to evaluate the antiproliferative or cancer cell killing effects of candidate drugs in tumor cell lines *in vitro*. [6] The precise mechanism of action of the candidate drugs was not critical to the selection process, and for many agents (e.g., doxorubicin) the mechanism of action was not defined until after the drugs were in widespread clinical use. This non-mechanistically based screening method

identified drugs that are cytotoxic and nonselective. As a result, most conventional anticancer drugs produce substantial clinical toxicity.

In clinical drug development, the initial dose-finding (phase I) clinical trials define the maximum tolerated dose, which is based on the severity of toxicity, as the optimal dose, rather than using a therapeutic endpoint to establish the optimal dose. This design is based on the premise that the highest tolerable dose will produce the maximum achievable cancer cell kill. In subsequent phase II trials, the maximum tolerated dose is evaluated in small cohorts of patients with different types of cancer to establish whether the drug has activity, which is defined as a greater than or equal to 50% decrease in the size of measurable tumors in at least 20% to 30% of patients with a specific type of cancer.[7]

Conventional front-line treatment regimens for most types of childhood cancer are composed of multiple anticancer drugs that are administered at their maximum tolerated dose intensity, even though these regimens typically produce substantial toxicity. Methods of rescuing or circumventing anticancer drug toxicity, such as the administration of hematopoietic growth factors and bone marrow or stem cell transplant to alleviate hematologic toxicity, have been incorporated into treatment regimens to allow for administration of higher doses of anticancer drugs.

The basic principles that guide the current use of cancer chemotherapy in pediatric and medical oncology are based on the goal of curing patients by eradicating all cancer cells and on empiric observations made in early clinical trials involving children with drug-sensitive cancers, such as acute lymphoblastic leukemia (ALL), Burkitt's lymphoma, and Wilms' tumor.[8] These principles include the use of multidrug combination regimens (i.e., combination chemotherapy), the administration of chemotherapy before the development of clinically evident metastatic disease (i.e., adjuvant chemotherapy), and the administration of drugs at the maximally tolerated dose rate (i.e., dose intensity).

**Combination Chemotherapy**

The importance of administering anticancer drugs in combination regimens was first appreciated in the treatment of ALL. Compared with single-agent therapy, the use of drug combinations significantly increased the percentage of patients achieving complete remission and prolonged the duration of their remissions.[9] At best, only 60% of patients treated with a single agent achieved complete remissions, but standard four- and five-drug combination induction regimens achieve complete remission rates that approximate or exceed 95%. Almost all patients on single-agent therapy experienced a relapse within 6 to 9 months, despite continuation of therapy with the same drug. Long-term remissions and cures were only attained after the institution of combination chemotherapy that incorporated the most active single agents.

The primary scientific rationale for the use of combination chemotherapy is to overcome drug resistance to individual agents, the incidence of which can often exceed 50% even in newly diagnosed cancers.[10,11] Because it is not feasible to predict whether a particular patient's tumor will respond to a given drug, administering anticancer drugs in combination ensures a greater chance of achieving a response (i.e., exposing the tumor to at least one active agent). In addition to providing a broader range of coverage against naturally resistant tumor cells, combination chemotherapy also may prevent or delay the development of acquired resistance in initially responsive tumors and provide additive or synergistic cytotoxic effects if agents with different mechanisms of action are selected.

A thorough knowledge of the clinical pharmacology of individual anticancer drugs is required to design effective combination chemotherapy regimens. Traditionally, combination chemotherapy regimens contain drugs with demonstrated single-agent activity against the type of tumor being treated, with a preference for agents that produced complete responses in patients with advanced or recurrent disease; drugs that are non–cross-resistant to overlap against drug-resistant subpopulations of tumor cells; drugs with non-antagonistic (i.e., additive or synergistic) mechanisms of action; and drugs with non-overlapping toxicity profiles, allowing each agent to be administered at its optimal dose and schedule.

**Adjuvant Chemotherapy**

Anticancer drugs are most effective when administered in the adjuvant setting to patients who are without evidence of residual disease after local therapy with surgery or radiation but who are at high risk to relapse at metastatic sites. Before the routine use of adjuvant chemotherapy, relapse at metastatic sites occurred in 60% to 95% of children with localized solid tumors after local therapy. The aim of adjuvant chemotherapy is to prevent metastatic recurrence by eliminating micrometastatic tumor deposits that are present at the time of diagnosis in the lungs, bone, bone marrow, lymph nodes, or other sites.[12] Adjuvant chemotherapy is efficacious for most of the common pediatric cancers, including Wilms' tumor, Ewing's sarcoma, lymphoma, rhabdomyosarcoma, astrocytoma, and osteosarcoma (Table 10-1).[13,14,15,16,17,18 and 19]

| Tumor | Adjuvant therapy | Survival (%) | |
|---|---|---|---|
| | | Without adjuvant chemotherapy | With adjuvant chemotherapy |
| Wilms' tumor | Vincristine, dactinomycin ± doxorubicin | 40 | 90 |
| Ewing's sarcoma | Vincristine, dactinomycin, cyclophosphamide | 5 | 50–60 |
| Lymphoma | CHOP, COMP, $CA_{1-5}$ | <10 | 50–90 |
| Rhabdomyosarcoma | Vincristine, dactinomycin, cyclophosphamide | 10–20 | 60 |
| Osteosarcoma | HDMTX, doxorubicin, cisplatin, BCD | 15 | 60 |
| Astrocytoma | Prednisone, vincristine, lomustine | 20 | 40 |

C, cyclophosphamide; H, doxorubicin; O, vincristine; M, methotrexate; P, prednisone; HDMTX, high-dose methotrexate; B, bleomycin; D, dactinomycin; $CA_{1-5}$, ten-drug regimen.
From Berg SL, Cortes EA, Delaney TF, Balis FM: Principles of treatment of pediatric solid tumors. Pediatr Clin North Am 1991;38:1249.

**TABLE 10-1. BENEFICIAL EFFECTS OF ADJUVANT CHEMOTHERAPY ON SURVIVAL OF PATIENTS WITH COMMON FORMS OF CHILDHOOD CANCER**

Theoretical considerations and experimental evidence support the use of adjuvant chemotherapy.[20,21 and 22] Microscopic foci of tumor should be more chemosensitive on a cell-kinetic basis, because a larger fraction of the cells are actively proliferating and potentially susceptible to the cytotoxic effects of the drugs. The smaller burden of tumor cells also implies a lower probability that drug-resistant cells are present. The mathematical modeling experiments of Goldie and Coldman, which assume that a curable tumor is one with no drug-resistant tumor cells and that the development of drug resistance is the result of a random genetic event, predict that the chance for cure is maximized if all available active drugs are given simultaneously in the adjuvant setting when there is minimal residual disease, and the probability that drug-resistant cells are present is low.[10,23]

Clinical experience has demonstrated a correlation between low tumor burden and the efficacy of chemotherapy.[22,23 and 24] Children presenting with extensive or disseminated tumors are less likely to be cured than children with the identical type of cancer but with a low tumor burden. For example, the event-free survival for patients with metastatic Ewing's sarcoma treated on the European Cooperative Ewing's Sarcoma Studies was 27% compared with 60% for patients presenting with localized disease.[25]

The selection of appropriate drugs and the optimal timing of drug therapy relative to the definitive local therapy are important considerations in the design of successful adjuvant chemotherapy regimens. Traditionally, drugs have been selected based on their activity in advanced disease. Animal models and clinical experience have shown that regimens producing the most dramatic responses in metastatic or recurrent disease have the greatest likelihood of being curative in the adjuvant setting.[24]

Adjuvant chemotherapy should begin as soon as possible after definitive local therapy. A delay to allow for recovery from surgery or radiation therapy may compromise the chance of curing the patient. One strategy to avoid delays caused by potential adverse interactions between chemotherapy and surgery or irradiation is the administration of the drug therapy before definitive local therapy. This approach, called *primary* or *neoadjuvant chemotherapy*, may also improve local control of the primary tumor by shrinking the primary and making it more amenable to surgical resection, in addition to providing earlier therapy for micrometastases.[26,27]

**Dose Intensity**

Most anticancer drugs have a steep dose-response curve, and a small increment in the dose can significantly enhance the therapeutic effect of a drug in preclinical studies. In animal tumor models, a twofold increase in the dose of cyclophosphamide can result in a tenfold increase in tumor cell killing. [28,29] Retrospective clinical studies have also demonstrated a relationship between dose intensity of anticancer drugs and disease outcome, but this relationship has not been consistently confirmed in randomized prospective trials.

In a meta-analysis (i.e., a compilation of data from multiple clinical trials) of chemotherapeutic regimens containing cyclophosphamide, methotrexate, and fluorouracil for metastatic breast cancer, Hryniuk and Bush observed a strong correlation between response rate and the relative dose intensity of the various regimens. [30] The relative dose intensity is calculated by normalizing the dose rate (mg per $m^2$ per week) for each agent to the dose rate in an arbitrarily selected standard regimen and then averaging the relative dose intensities for all agents in the regimen to derive the relative dose intensity for the regimen. Over a threefold range in relative dose intensity, the response rate in metastatic breast cancer ranged from 12% to 84%. Retrospective meta-analyses in stage II breast cancer, ovarian cancer, colorectal cancer, and lymphoma have also demonstrated a correlation between the dose intensity of the drug regimen and disease outcome. [31,32] However, prospective randomized trials have failed to demonstrate a survival advantage for more dose-intensive regimens, including high-dose chemotherapy with bone marrow or stem cell rescue, compared with standard dose regimens in breast, ovarian, and small cell lung cancers [33,34 and 35] and for more dose-intensive cisplatin in germ cell tumors.[36]

For children with ALL and osteosarcoma, relapse rates are significantly lower in patients receiving more dose-intense chemotherapy. [37,38,39,40 and 41] In a randomized trial, patients with ALL receiving standard doses of methotrexate and mercaptopurine had a median survival of 15 months compared with 6 months for the group randomized to a half-dose maintenance regimen.[37] In children with high-risk ALL, those who received less than 94% of the protocol-prescribed dose of vincristine, anthracycline, and L-asparaginase during intensification therapy were 5.5 times more likely to experience a subsequent adverse event than patients who received at least 99% of the prescribed dose of these agents.[38] Oral mercaptopurine dose intensity during maintenance therapy is also predictive of event-free survival in ALL. [39] However, in the latter study, lower mercaptopurine dose-intensity was primarily the result of missed doses rather than reductions of the daily dose, leading the authors to conclude that prescribing higher doses of mercaptopurine could be counterproductive if greater hematologic toxicity resulted in treatment delays.

Retrospective analyses of osteosarcoma trials demonstrated a twofold higher relapse rate in patients receiving less than 75% of their recommended dose of chemotherapy compared with patients receiving 75% or more in one study[40] and a threefold higher relapse rate in a second study using 80% of the protocol prescribed dose as a cutoff.[41]

Meta-analyses, such as those performed by Hryniuk and Bush, have also been performed for several pediatric tumors. [42,43] Analysis of 44 clinical trials involving 1,592 patients older than 1 year of age with stage IV neuroblastoma revealed a fivefold to tenfold range in the dose intensity of the individual agents studied. [42] The dose intensity of four drugs (i.e., teniposide, cisplatin, cyclophosphamide, and doxorubicin) significantly correlated with response and survival. Similarly, examination of the relation between individual drug dose intensities and disease outcome in osteosarcoma and Ewing's sarcoma suggests that doxorubicin dose intensity is an important determinant of response in osteosarcoma and disease-free survival of patients with Ewing's sarcoma.[43] The dose intensities of the other agents in the combination regimens used to treat these tumors did not appear to be as strongly correlated with disease outcome, suggesting that future combination regimens should be designed to optimize the dose intensity of doxorubicin.

Prospective randomized trials to assess the importance of dose intensity in childhood cancers have not been systematically performed for most tumor types. The administered dose intensity of dactinomycin and doxorubicin in pulse-intensive regimens for Wilms' tumor was significantly higher than for the standard treatment regimens, but there was no survival advantage associated with the enhanced dose intensity. [44,45] In a randomized trial of Filgrastim in children with high-risk ALL, the treatment interval was shorter with Filgrastim, resulting in a slight increase in dose intensity but no impact on event-free survival. [46]

Methods for maximizing dose intensity include greater patient and physician willingness to tolerate drug toxicities; more aggressive supportive care of patients experiencing these side effects; selective rescue of the patient from toxicity, such as with bone marrow or peripheral stem cell transplantation or the administration of colony-stimulating factors, such as granulocyte colony-stimulating factor (G-CSF) the use of regional chemotherapy (e.g., intra-arterial or intrathecal delivery) to achieve high drug concentrations at local tumor sites while minimizing systemic drug exposure; and the development of new treatment schedules, such as long-term continuous infusions that may allow more drug to be administered over a given period.

## CLINICAL PHARMACOLOGY OF ANTICANCER DRUGS

The primary role of the pediatric oncologist is to orchestrate the administration of complex combination chemotherapy regimens to children in the setting of multimodal (i.e., surgery, radiotherapy, and chemotherapy) therapy. Special care must be taken because the anticancer drugs used in these regimens have the lowest therapeutic index of any class of drugs and predictably produce significant, even life-threatening toxicity at therapeutic doses ( Fig. 10-2).[47] However, implementing significant dose reductions or delays in therapy to attenuate these toxicities may compromise the therapeutic effect and place the patient at an increased risk for disease recurrence, a uniformly fatal event with most childhood cancers. The cancer chemotherapist must carefully balance the risks of toxicities from therapy against the risk of tumor recurrence from inadequate treatment. The crucial adjustments in the dose and schedule of chemotherapy needed to achieve this balance often must be made empirically, however, because therapeutic drug monitoring for most agents is not available.

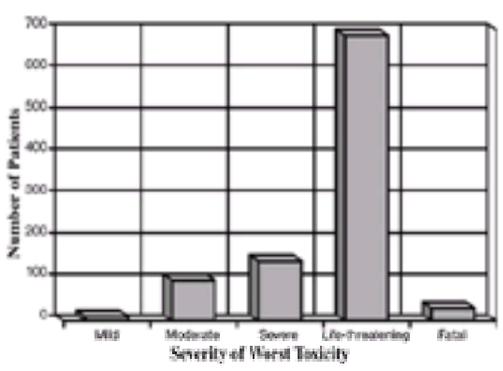



**FIGURE 10-2.** The worst degree of any toxicity experienced by patients (n = 1,062) treated on one of the eight treatment arms of the Intergroup Rhabdomyosarcoma Study III. Seventy-eight percent of patients had at least one severe or life-threatening toxicity, and there were 32 toxicity-related deaths. (Adapted from Table 6 in Crist W, Gehan EA, Ragab A, et al. The third Intergroup Rhabdomyosarcoma Study. J Clin Oncol 1995;13:610.)

To ensure that these drugs are used safely and effectively, the pediatric oncologist must have an in-depth knowledge of the clinical pharmacology of these agents, including the mechanisms of drug action, pharmacokinetics (e.g., absorption, distribution, metabolism, and elimination), spectrum of toxicities, potential drug interactions, and mechanisms of drug resistance.

### Mechanism of Action

Although advances in basic research have provided profound insights into the pathogenesis of many forms of childhood cancer and offer hope for the development of specific and selective new cancer treatments,[48,49,50,51,52 and 53] most current conventional anticancer drugs have nonselective mechanisms of action that target vital macromolecules (e.g., DNA) or metabolic pathways that are critical to both malignant and normal cells; and as a result they cause many undesirable and potentially severe toxic effects (Fig. 10-2).

Most anticancer drugs produce their cytotoxic effects by interfering at some stage with the synthesis or function of the vital nucleic acids, DNA and RNA ( Fig. 10-3). For example, the alkylating agents are chemically reactive compounds that damage DNA by covalently bonding to and crosslinking nucleobases within the DNA, [54] and

the antimetabolites block the synthesis of nucleotide precursors or are directly incorporated into DNA as fraudulent bases. The topoisomerases are also an important target of anticancer drugs. These nuclear enzymes maintain the three-dimensional structure of DNA and are critical for DNA replication, transcription, repair, and recombination. The topoisomerases work by cleaving and religating DNA, and agents such as the anthracyclines, epipodophyllotoxins, and camptothecins interfere with religation, resulting in protein-associated DNA strand breaks. [55,56,57] and [58]

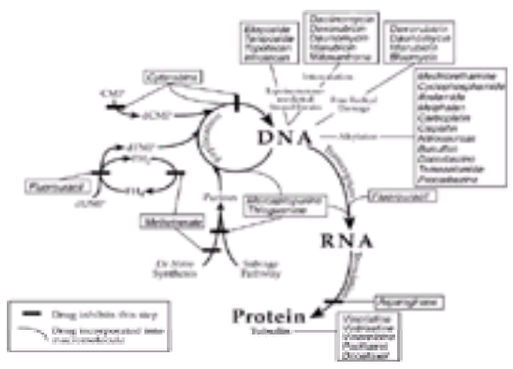



**FIGURE 10-3.** Site of action of the commonly used anticancer drugs. CMP, cytidine monophosphate; dCMP, deoxycytidine monophosphate; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; $FH_2$, dihydrofolate; $FH_4$, tetrahydrofolate.

Dysregulation of the cell cycle and programmed cell death (apoptosis) are common to all forms of cancer. [59,60] and [61] These cellular processes are genetically controlled, and mutations to genes involved in these highly complex and interrelated pathways can result in loss of control of DNA replication and cell division and suppression of the apoptotic response to receptor-linked or DNA damage-induced signals. In addition to their role in tumorigenesis, mutations in cell cycle regulatory genes and genes involved in apoptosis may modulate the sensitivity of cancer cells to anticancer drugs. [59,61,62] and [63] The cellular damage produced by most anticancer drugs appears to induce apoptosis in chemosensitive cancer cells. Overexpression of oncogenes that promote apoptosis, such as C- *myc* and N-*myc*, can enhance the chemosensitivity of tumor cells, whereas overexpression of Bcl-2, which blocks the apoptotic pathway, can attenuate drug-induced apoptosis and convey pleiotropic resistance to anticancer drugs. [64,65]

The cell cycle is regulated by negative feedback controls or checkpoints that block the cell from proceeding to the next cell cycle event until the prior event is completed and until needed repairs to DNA are performed. [66,67,68] and [69] Normal cells are arrested in $G_1$ phase in response to DNA damage caused by cytotoxic drugs, allowing for repair of the DNA damage. [70,71] If this DNA damage is not repaired, the complex process of DNA replication and cell division is disrupted. Mutations in cell cycle regulatory genes that have been implicated in tumorigenesis most frequently involve genes controlling the transition from the $G_1$ to S phases of the cell cycle, [59] and loss of checkpoint function (e.g., p21) can enhance chemo- and radiosensitivity. [59,72] The same mechanisms that play a role in the pathogenesis of cancer could also sensitize the cancer cell to DNA-damaging anticancer drugs.

p53 mutations and loss of p53 function, which occur in one-half of all human cancers, have been associated with enhanced or decreased sensitivity to anticancer drugs in preclinical studies. [73,74,75] and [76] The role of p53 as a dual-effect regulator that induces apoptosis in its activated state and regulates the cell cycle at the $G_1$/S and $G_2$/M checkpoints may be responsible for these seemingly contradictory results. Clinical studies correlating p53 mutational status to disease outcomes, such as response and survival, have also failed to consistently demonstrate a relationship. [76]

An understanding of the mechanism of drug action is useful in predicting which tumors may respond to the drug based on their biochemical and cytokinetic profiles and which drug combinations may produce additive or synergistic antitumor effects. Combining agents that together could enhance the inhibition of vital intracellular processes through sequential or concurrent blockade or lead to complementary inhibition of specific metabolic pathways has been a traditional strategy for the design of combination regimens. [77] A drug's schedule of administration may also be influenced by its mechanism of action. For example, the antimetabolites, which are inhibitory only during S phase in the cell cycle, tend to be more cytotoxic if administered by prolonged infusion. This approach ensures that a greater number of tumor cells are exposed to the drug as they pass through S phase.

## Pharmacokinetics

The discipline of pharmacokinetics deals with quantitative aspects of drug disposition in the body, including drug absorption, distribution, biotransformation (metabolism), and excretion (Table 10-2). Although the pharmacokinetic behavior of most of the commonly used anticancer drugs has been studied in adults, many of these agents have not been extensively evaluated in children. As the technology to measure the concentration of these drugs and their metabolites in biologic fluids has improved, a greater emphasis has been placed on studying anticancer drug pharmacokinetics.

| Term | Common abbreviation | Units | Definition |
|---|---|---|---|
| Clearance | Cl | Volume (mL/min) | Used to quantify the rate of drug elimination; expressed in terms of volume of plasma cleared of drug per unit of time. Total clearance is the sum of renal, metabolic, spontaneous chemical degradation, and biliary (fecal) elimination. |
| Half-life | $t_{1/2}$ | Time (h) | Time required to reduce the drug concentration by 50%. Plasma drug disappearance frequently has multiple phases with differing rates of disappearance (e.g., rapid distribution phase, terminal or elimination phase). Half-lives listed for drugs in this chapter are the postinfusion (terminal, elimination) half-lives, unless otherwise noted. |
| Area under the curve | AUC | Conc. × time (μM•h) | Quantitates total drug exposure; integral of drug concentration over time or the area under the plasma concentration-time curve; used in calculation of clearance and bioavailability. |
| Volume of distribution | Vd, $V_{ss}$ | Volume (L) | Relates plasma concentration to total amount of drug in the body (i.e., volume required to dissolve the total amount of drug to give the final concentration found in plasma); a property of the drug rather than a real volume or physiologic compartment. |
| Bioavailability | F | Fraction (%) | Rate and extent of absorption of a drug, frequently synonymous with the fraction of a dose absorbed when administered by some route other than intravenous. |
| Biotransformation | — | — | Enzymatic metabolism of a drug; may result in the activation of a prodrug, conversion to other biologically active intermediates, or inactivation of a drug. |

Conc., concentration.

**TABLE 10-2. PHARMACOKINETIC TERMS**

Pharmacokinetic studies have revealed substantial interpatient variability in drug disposition and systemic drug exposure with most anticancer drugs. [78,79] Administering a standard dose of etoposide, doxorubicin, or cyclophosphamide to a group of children results in a twofold to tenfold range in systemic drug exposure, as measured by the area under the plasma drug concentration-time curve (AUC), [80] and substantial variability in systemic drug exposure is also observed with orally administered agents such as methotrexate and mercaptopurine. [81] Assuming that drug effect is more closely related to systemic drug exposure than dose, these differences in drug disposition could account for the variability in toxicities and responses observed with most combination chemotherapy regimens using standardized doses of individual agents. [82] Variability in anticancer drug disposition in children may result from age-related developmental changes in body composition and excretory organ function, variation in rate of metabolism and excretion of drug by the kidneys or liver, variation in the extent of drug-protein binding, drug interactions, and pharmacogenetics. [78,83,84,85,86,87,88] and [89]

The most important determinant of variability in anticancer drug pharmacokinetics is the rate of drug metabolism. Enzymatically catalyzed biotransformation of lipophilic drugs and other xenobiotics usually yields more hydrophilic (water soluble) metabolites that have lost their pharmacologic activity and that are more readily excreted in urine or feces. [90] Drug metabolizing enzymes are divided into two groups based on the type of reaction that they catalyze. Phase I reactions (e.g., oxidation, hydrolysis, reduction, and demethylation) introduce or expose a functional group (e.g., hydroxyl group) on the drug. Phase I reactions usually diminish the drug's pharmacologic activity, but some prodrugs, such as cyclophosphamide, are converted to active metabolites by these enzymes. Phase II conjugation reactions covalently link a highly polar conjugate (e.g., glucuronic acid, sulfate, glutathione, amino acids, or acetate) to the functional group created by the phase I reaction. The

conjugated drugs are highly polar, usually devoid of pharmacologic activity, and rapidly excreted.

The cytochrome P450 (CYP) superfamily of enzymes catalyze oxidation and demethylation reactions for a wide spectrum of drugs and xenobiotics. These heme-containing proteins, which are membrane-bound and typically reside in the endoplasmic reticulum, have very broad and overlapping substrate specificity. [91] The CYP enzymes are categorized into families and subfamilies according to their amino acid sequence similarity. Sequences that are more than 40% identical belong to the same family (e.g., CYP1) and sequences that are more than 55% identical are in the same subfamily (e.g., CYP1A).[92] Subfamilies may contain multiple isoforms (e.g., CYP1A2). More than 1,000 CYP sequences have been identified across all species. Humans have at least 17 families and 39 subfamilies of CYP genes. The CYP1, CYP2, and CYP3 families are primarily responsible for hepatic drug and xenobiotic metabolism in humans, and CYP3A is the most important subfamily, accounting for the metabolism of half of all drugs.[92,93] CYP3A4 is the most abundant P450 enzyme in the liver, and it is known to metabolize more than 50 different drugs. Genetic, environmental, physiologic, and developmental factors contribute to individual differences in the rate of drug metabolism by the CYP enzymes. [90,94] A number of CYP genes are known to be genetically polymorphic, and, in some cases, these alterations can influence enzyme activity and drug metabolism phenotype.[95,96] Exposure to certain drugs or environmental chemicals can induce the expression of specific CYP enzymes or inhibit CYP enzyme activity. [91] For example, members of the CYP3A subfamily are induced by rifampin, barbiturates, phenytoin, and dexamethasone, and this enzyme induction can enhance the rate of metabolism of other drugs that are substrates for CYP3A enzymes. CYP3A enzymes are inhibited by macrolide antibiotics and azole antifungal drugs, which can block the metabolism of other CYP3A substrates.[93]

Pharmacogenetic variation in the level of expression of other non-P450 drug-metabolizing enzymes is an important determinant of toxicity for mercaptopurine (thiopurine methyl transferase),[97,98,99] and[100] fluorouracil [dihydropyrimidine dehydrogenase (DPD)],[101] and the investigational agent amonifide (acetylator status).[102]

The significant interpatient variation in systemic drug exposure with current dosing methods, the toxic nature of these agents, and the potential importance of dose intensity in cancer chemotherapy point to the need for more precise, individualized dosing methods for anticancer drugs,[79,85,86,103,104] such as the adaptive dosing techniques that have been successfully applied to individualize carboplatin dose [105] and therapeutic drug monitoring of methotrexate that plays a critical role in determining the duration of leucovorin rescue after high-dose methotrexate therapy.[79,98,104] A prerequisite for these individualized dosing methods is the establishment of the relation between a drug's pharmacokinetics and pharmacodynamics (toxicity or therapeutic effect). Systemic drug exposure (AUC) of anticancer drugs is usually the best correlate of the drug's toxic or therapeutic effects. However, this requires plasma sampling at multiple times over a prolonged period, which may not be practical for monitoring large numbers of patients. Through pharmacokinetic modeling, a limited number of sampling times that can reliably estimate the AUC can often be identified, providing a more practical pharmacokinetic monitoring schedule.[79,106,107] Parameters other than AUC, such as peak or trough concentration or average steady-state concentration, can also be evaluated for clinical correlations.

Even though therapeutic drug monitoring has yet to play a significant role in the day-to-day management of the patient with cancer, the pharmacokinetic parameters are important for determining the optimal dose, schedule, and route of administration of the drug. Knowledge of the route of elimination of a drug is also helpful in adjusting the dosage for patients with hepatic or renal dysfunction.[108,109] and[110]

Physiologic differences between children and adults can affect drug disposition and must be considered in determining the appropriate dose and schedule of the drug for children. Developmental differences in drug absorption, plasma protein or tissue binding, functional maturation of excretory organs, and distribution of drug in the various tissues of the body (Table 10-3) can result in differences in systemic drug exposure for children compared with adults treated with the same dose.[83,84] The most dramatic changes in excretory organ function and body composition occur during the first few days to months of life, but there are very limited data on the disposition of anticancer drugs in infants (younger than 1 year old).[111]

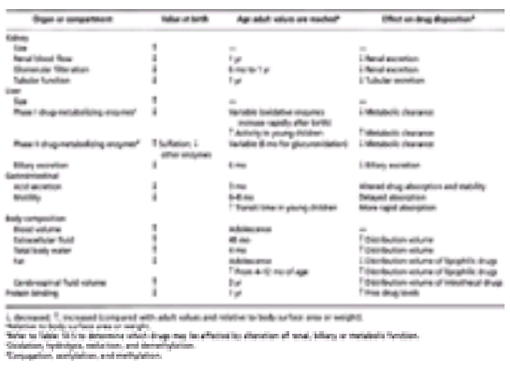


**TABLE 10-3. PHYSIOLOGIC DIFFERENCES IN CHILDREN THAT MAY INFLUENCE DRUG DISPOSITION**

## Toxicity

In therapeutic doses, actively dividing normal host cells, such as those in the bone marrow or the mucosal epithelium, are sensitive to the cytotoxic effects of anticancer drugs.[112] The nonselective mechanisms of action and resulting low therapeutic indices of these agents mean that a high incidence of potentially severe toxicities must be tolerated to administer effective doses.[113,114] and[115] Acute toxicities common to many of the anticancer drugs include myelosuppression, nausea and vomiting, alopecia, orointestinal mucositis, liver function abnormalities, allergic or cutaneous reactions, and local ulceration from subcutaneous drug extravasation. These acute toxicities occur over hours to weeks after a dose and are usually reversible. Many drugs also have unique toxicities affecting specific organs or tissues, such as cardiotoxicity associated with the anthracyclines; hemorrhagic cystitis associated with cyclophosphamide and ifosfamide; peripheral neuropathy from vincristine, cisplatin, and paclitaxel; nephrotoxicity from cisplatin and ifosfamide; ototoxicity from cisplatin; and coagulopathy from L-asparaginase. Many of these latter toxicities are cumulative (i.e., occur after multiple doses), and in some cases they are not completely reversible (e.g., anthracycline cardiotoxicity).

A significant portion of an oncologist's time is spent in providing supportive care for patients experiencing acute and long-term drug toxicities. [116] A number of therapeutic approaches have evolved to attenuate these toxicities, to make the therapy more tolerable, and to safely increase the dose intensity of regimens by circumventing dose-limiting toxicities.[112,117,118] and[119] Bone marrow or peripheral stem cell transplantation to rescue patients from myeloablative doses of anticancer drugs is an example of this rescue approach. Other widely used forms of rescue include the administration of leucovorin or carboxypeptidase-G $_2$[120] to counteract the toxicities of high-dose methotrexate, the use of antiemetics to block nausea and vomiting,[121,122] and[123] the use of mesna to prevent the hemorrhagic cystitis caused by the oxazaphosphorines,[124] the use of colony-stimulating factors (e.g., G-CSF or erythropoietin) to alleviate myelosuppression,[125,126,127] and[128] and the use of dexrazoxane (ICRF-187) to prevent anthracycline cardiotoxicity.[129,130]

Amifostine is a broad-spectrum cytoprotective agent that protects many normal tissues and organs, including bone marrow, kidney, lung, orointestinal mucosa, and peripheral nerves, against the toxic effects of radiation and a variety of anticancer drugs, such as the alkylating agents, the anthracyclines, and platinum compounds.[131,132,133,134,135,136,137] and[138] Amifostine is a prodrug that is converted by alkaline phosphatase to WR-1065, which is a free sulfhydryl compound that scavenges free radicals and binds to active metabolites of anticancer drugs.[134] Preferential conversion of amifostine to WR-1065 and uptake of WR-1065 in normal tissues compared to tumors account for the selective nature of the rescue.[135] Preliminary clinical trials of amifostine have been conducted in children,[139,140] but its role in treating childhood cancers has yet to be defined.

The toxicity of anticancer drugs has a major impact on the dosing of these agents. The endpoint of the phase I dose-finding studies for most anticancer drugs is the identification of the maximum-tolerated dose, which is considered the optimal dose. The dosing interval (every 21 to 28 days) for anticancer drugs is determined by the duration of acute toxicities, and dose modifications are usually based on the severity or duration of toxicities on the prior treatment cycle. The life-time cumulative dose of the anthracyclines and bleomycin is limited to prevent cardiotoxicity and pulmonary toxicity. This toxicity-based dosing approach for anticancer drugs reflects the lack of data on the relationship between dose and anticancer effect.

The severity, incidence, and time course of toxicities are important factors in designing optimal drug combinations or adjusting doses to avoid overlapping toxicities. For example, nonmyelosuppressive agents, such as vincristine, prednisone, L-asparaginase, and high-dose methotrexate with leucovorin rescue, often can be administered with traditional myelosuppressive drugs without compromising the dose of either agent. Some combinations have administered nonmyelosuppressive agents during the period of marrow suppression from myelotoxic drugs to ensure continuous exposure of the tumor to cytotoxic therapy. [141,142]

The long-term side effects of cancer chemotherapy are also of particular concern to the pediatric oncologist because of the high cure rates and the long life spans of successfully treated patients. The adverse late effects of chemotherapy on growth, development, and reproductive function; possible permanent cardiac, pulmonary, or renal damage; and possible carcinogenic and teratogenic effects are discussed in Chapter 49.

**Drug Interactions**

In addition to being administered in combination regimens, the anticancer drugs are also administered with antiemetics, antibiotics, analgesics, stool softeners, and other agents used to alleviate the side effects of chemotherapy or the underlying cancer. This degree of polypharmacy introduces a significant risk of drug interactions.[87,143,144 and 145] Clinicians must be aware of potential drug interactions, which can alter the disposition of anticancer drugs or alter their effects at the target site in tumor or normal tissues, to avoid unexpected or severe toxicities or antagonism that can diminish a drug's antitumor effect. [87,143] However, little information is available about drug interactions involving the anticancer drugs with one another or with other classes of drugs. To some extent, the already high incidence of toxicities and treatment failures and the continued reliance on empirical dosing methods that do not include routine therapeutic drug monitoring obscure the recognition of possible interactions.

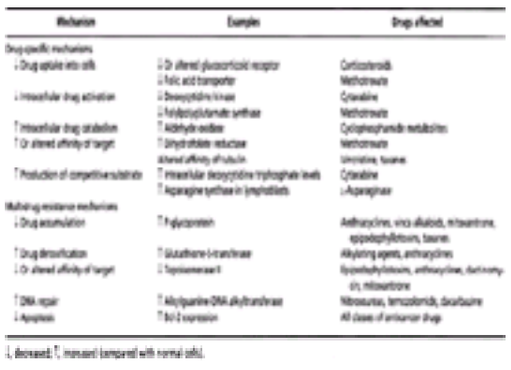
In some instances, drug interactions have been exploited to increase the antitumor effects of the anticancer agent being administered, such as with the combination of fluorouracil and leucovorin.

**Drug Resistance**

Although toxic effects of anticancer drugs are usually predictable, the response of any given tumor to individual agents is not. Clinical resistance to anticancer drugs is the primary reason for treatment failure in childhood cancers. Drug resistance can be present at the outset of treatment or can become clinically apparent under the selective pressure of drug exposure.[146] The magnitude of the problem of drug resistance was appreciated early in cancer chemotherapy for childhood cancers. Less than one-half of children with ALL who were treated with single-agent therapy achieved a complete remission, and almost all of the patients who did respond eventually relapsed despite continuation of the drug that produced the remission. [8,147]

The development of most forms of drug resistance has a genetic basis.[10,148] The inherent genetic instability of tumor cells results in the spontaneous generation of drug-resistant clones as a consequence of a mutation, deletion, gene amplification, translocation, or chromosomal rearrangement. [10,148] These genetic alterations are presumed to be random events, which may account for the variability in response observed in most clinical trials. This genetic basis for drug resistance means that resistance can be inherited by subsequent generations of tumor cells, and under the selective pressure of drug exposure, drug-resistant cancer cells become the predominant subpopulation. At a biochemical level, there are a variety of mechanisms by which tumors become drug resistant. In most cases, these alterations in cellular metabolism can be related to an increase, a decrease, or an alteration in some gene product, such as the gene amplification identified in methotrexate-resistant cells that results in overproduction of dihydrofolate reductase (DHFR), the target enzyme of methotrexate. [149]

Genetically-based molecular or biochemical alterations in cancer cells can produce anticancer drug resistance that is specific to a single agent or class of agents or provides protection from a broad range of anticancer drugs ( Table 10-4). In the latter form of resistance, termed *multidrug resistance*, a single cellular alteration conveys resistance simultaneously to multiple unrelated drugs, including drugs to which the cancer has not been exposed. [150,151 and 152] The best studied multidrug-resistant phenotype is associated with decreased intracellular drug accumulation and an increase in a plasma membrane, adenosine triphosphate–dependent drug efflux pumps, such as P-glycoprotein (P-gp), or the family of multidrug resistance proteins (MRPs). [151,153,154,155,156,157,158,159,160 and 161]



| Mechanism | Examples | Drugs affected |
|---|---|---|
| Drug-specific mechanisms | | |
| ↓ Drug uptake into cells | ↓ Or altered glucocorticoid receptor | Corticosteroids |
| | ↓ Folic acid transporter | Methotrexate |
| ↓ Intracellular drug activation | ↓ Deoxycytidine kinase | Cytarabine |
| | ↓ Polyglutamate synthase | Methotrexate |
| ↑ Intracellular drug catabolism | ↑ Aldehyde oxidase | Cyclophosphamide metabolites |
| ↑ Or altered affinity of target | ↑ Dihydrofolate reductase | Methotrexate |
| | Altered affinity of tubulin | Vincristine, taxanes |
| ↑ Production of competitive substrate | ↑ Intracellular deoxycytidine triphosphate levels | Cytarabine |
| | ↑ Asparagine synthase in lymphoblasts | L-Asparaginase |
| Multidrug resistance mechanisms | | |
| ↓ Drug accumulation | ↑ P-glycoprotein | Anthracyclines, vinca alkaloids, mitoxantrone, epipodophyllotoxins, taxanes |
| ↑ Drug detoxification | ↑ Glutathione S-transferase | Alkylating agents, anthracyclines |
| ↓ Or altered affinity of target | ↓ Topoisomerase II | Epipodophyllotoxins, anthracyclines, dactinomycin, mitoxantrone |
| ↑ DNA repair | ↑ Alkylguanine DNA alkyltransferase | Nitrosoureas, temozolomide, dacarbazine |
| ↓ Apoptosis | ↑ Bcl-2 expression | All classes of anticancer drugs |

↓, decreased; ↑, increased (compared with normal cells).

**TABLE 10-4. MECHANISMS OF ANTICANCER DRUG RESISTANCE**

P-gp and the *mdr-1* gene that encodes for it are expressed in human tumor specimens, including ALL, neuroblastoma, rhabdomyosarcoma, neuroepithelioma, Ewing's sarcoma, retinoblastoma, and osteosarcoma,[162,163,164,165,166 and 167] and in a variety of normal human tissues, such as the biliary canaliculi in the liver, the proximal tubules in the kidney, the mucosal lining of the jejunum and colon, the adrenal gland, hematopoietic progenitor cells, and the endothelial cells of blood vessels within the central nervous system (CNS) and testis.[168] P-gp appears to be responsible for excretion of toxic compounds from these normal cells. [162,168,169] Although the presence of P-gp in tumor specimens has been associated with a worse prognosis and a poor response to therapy in some studies, the clinical significance of P-gp expression in childhood cancers remains controversial, in part because of the lack of a universal standard for quantifying expression at an RNA or protein level.[150,151,166,170]

Chemosensitizers, which are capable of reversing multidrug resistance caused by P-gp *in vitro*, are undergoing clinical testing. The initial agents that were tested included drugs with other, unrelated pharmacologic actions, such as the calcium channel blocker, verapamil, and the immunosuppressant, cyclosporine; toxicity prevented achievement of adequate concentrations to block P-gp in patients. Newer chemosensitizing agents, such as valspodar, which are being developed specifically as P-gp inhibitors, are in the initial phases of clinical testing in childhood cancers. If these chemosensitizers are demonstrated to enhance the efficacy of anticancer drugs in well-designed prospective trials, this would support a role for P-gp as a clinically significant mechanism of drug resistance. However, because these chemosensitizers also inhibit P-gp excretory function in normal tissues, such as the liver and kidney, they can interfere with the elimination of the anticancer drug, leading to enhanced systemic exposure and increased toxicity. [171,172 and 173] The potential for marked alteration in the pharmacokinetics of the anticancer drug when administered in combination with a chemosensitizing agent must be taken into consideration in the design and interpretation of clinical trials testing the efficacy of chemosensitizers.

The less well-studied MRP family of drug transporters also appear to have a broad substrate specificity, which includes anionic drugs, neutral drugs that are conjugated to glutathione, glucuronate, or sulfate, nucleoside analogs, and cisplatin. [160,161] P-gp inhibitors, such as valspodar, do not effectively block drug transport by MRP1 and MRP2. The role of these transporters in clinical resistance to anticancer drugs is under study.

Other mechanisms for multidrug resistance include an enhanced capacity to repair DNA damage produced by alkylating agents [152,174,175]; the detoxification of chemically reactive forms of alkylating agents and anthracyclines by glutathione [176]; decreased levels of topoisomerase II, the target enzyme of the anthracyclines, epipodophyllotoxins, and dactinomycin[177]; and suppression of apoptotic pathways.[60,61,65] The loss of DNA mismatch repair activity results in multidrug resistance by

impairing the cancer cell's ability to detect DNA damage and activate apoptosis. [178]

The mechanism of drug resistance is an important consideration in selecting agents to be included in combination regimens or as second-line therapy in relapsed patients. Ideally, drug combinations should be composed of non–cross-resistant agents, and relapse treatment regimens should avoid the use of drugs that are cross-resistant with drugs used in the front-line regimen. With advances in the understanding of the mechanisms of drug resistance, specific treatment approaches may be devised to prevent the development of or overcome drug resistance in tumor cells.

In the remainder of this chapter, the pharmacologic characteristics of the anticancer drugs used to treat childhood cancers are reviewed. Tables summarize the general pharmacologic properties (Table 10-5) and pharmacokinetic parameters (Table 10-6) of the commonly used anticancer drugs.

**TABLE 10-5. PHARMACOLOGIC PROPERTIES OF THE COMMONLY USED ANTICANCER DRUGS**

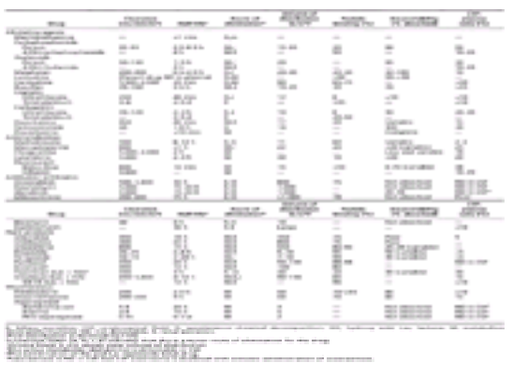


**TABLE 10-6. PHARMACOKINETIC PARAMETERS OF THE COMMONLY USED ANTICANCER DRUGS**

## ALKYLATING AGENTS

The alkylating agents have a broad range of clinical activity in childhood cancers. These drugs are chemically reactive compounds that exert their cytotoxic effect through the covalent bonding of an alkyl group to important cellular macromolecules ( Fig. 10-4).[54] Although a number of nucleophilic macromolecules and their precursors are potential targets for alkylation intracellularly, damage to the DNA template and the resulting induction of apoptosis appear to be the major determinants of cytotoxicity.[54,61,63,179,180] With the bifunctional alkylating agents that have two alkylating groups, this damage appears to result primarily from interstrand and intrastrand DNA-DNA and DNA-protein crosslinks.[179,181]

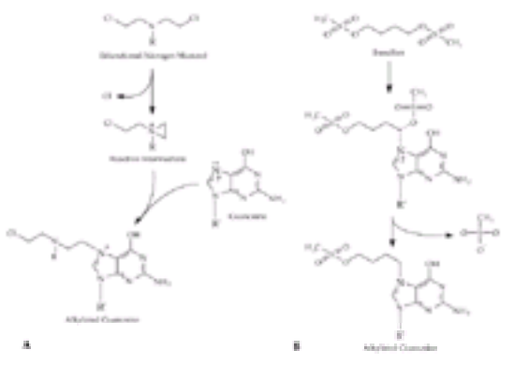


**FIGURE 10-4.** Mechanisms of alkylation of the nucleophilic $N^7$ position of guanosine. **A:** The bifunctional nitrogen mustard illustrates the $S_N1$ type of alkylation reaction, in which a reactive intermediate forms spontaneously and then rapidly reacts with the nucleophilic group. The rate-limiting step for $S_N1$ alkylation is the formation of the reactive intermediate, and thus the reaction exhibits first-order kinetics (i.e., independent of the target nucleophile concentration). If the second chloroethyl group also reacts with another nucleotide base, a crosslink is formed. **B:** Busulfan exemplifies an $S_N2$ reaction, characterized by a bimolecular nucleophilic displacement. In this case, the methylsulfonate group on either end of busulfan is displaced by the nucleophilic group on guanosine. The rate of $S_N2$ alkylation reactions depends on the concentration of the alkylating agent and the target nucleophile, and it therefore follows second-order kinetics.

Alkylating agents have steep dose-response curves in experimental model systems.[182] A log-linear relationship exists between tumor cell killing and the concentration of the alkylating agent, and this correlation is maintained through four to five orders of magnitude of cell killing. This steep dose-response relationship for alkylating agents provides a strong rationale for their use in high-dose therapy regimens. Because of the significant myelosuppressive effects of these drugs, high-dose alkylator therapy is generally administered in conjunction with bone marrow or peripheral stem cell transplantation to prevent permanent bone marrow aplasia. The use of melphalan and busulfan in childhood cancers is limited almost exclusively to high-dose transplantation preparative regimens, and other alkylating agents, such as cyclophosphamide and thiotepa, are also frequently incorporated into these regimens. [183,184 and 185]

Myelosuppression is the major dose-limiting toxicity for most of the commonly used alkylating agents. Other common acute toxic effects include nausea and vomiting, alopecia, allergic and cutaneous reactions, and gastrointestinal and neurologic toxicity at high doses. Of particular concern to the pediatric oncologist are the potential long-term effects of alkylator therapy. Alkylating agents can produce gonadal atrophy, permanently affecting reproductive function. The nitrogen mustards and the nitrosoureas have been linked to pulmonary fibrosis, and nephrotoxicity of the nitrosoureas, cisplatin, and ifosfamide can permanently impair renal function. [186,187] These agents are also highly carcinogenic, mutagenic, and teratogenic. [188,189]

The pharmacokinetics of the alkylating agents has been difficult to study, because the chemical reactivity and inherent chemical instability of the active alkylating species make their measurement in biologic fluids difficult. Spontaneous hydrolysis of alkylating agents or their active metabolites in solution can be a major route of

drug elimination. Most alkylating agents also undergo some degree of enzymatic metabolism, which can produce active and inactive metabolites. [190]

Several mechanisms for the development of resistance to alkylating agents have been described, including a decrease in drug uptake or transport by the cell; an increase in intracellular thiol compounds (glutathione) that are capable of detoxifying active alkylating species; enhancement of intracellular enzymatic catabolism to inactive metabolites; and an increase in the capability for repair of DNA damage produced by alkylation. [152,174,176,191,192,193,194,195,196] and [197] Loss of DNA mismatch repair capacity induces resistance to the methylating agents procarbazine and temozolomide, busulfan, and the platinum analogs. [178] *In vitro* studies indicate that resistance to alkylating agents is difficult to induce despite protracted exposure of cells to the drugs and that, after resistance has been induced, it often is not stable without drug in the medium to create continuous selection pressure. Cross-resistance to these drugs is not common in preclinical models. [198,199] and [200]

Of the various classes of alkylating agents, the nitrogen mustards and the nitrosoureas are most frequently used in the treatment of the childhood cancers. The chemical structures of the nitrogen mustards, the nitrosoureas, and several nonclassical alkylators are shown in Figure 10-5, Figure 10-7, Figure 10-9, and Figure 10-11).

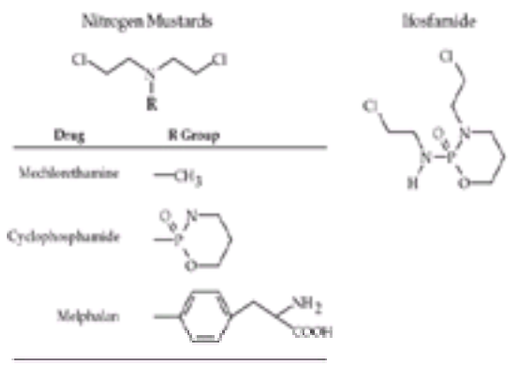



**FIGURE 10-5.** Chemical structures of the nitrogen mustard alkylating agents and the cyclophosphamide isomer, ifosfamide.

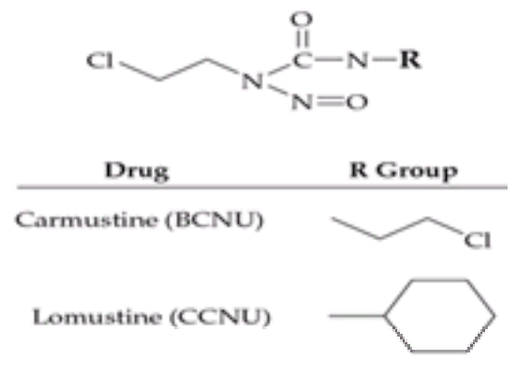



**FIGURE 10-7.** Chemical structures of the nitrosoureas, carmustine and lomustine.

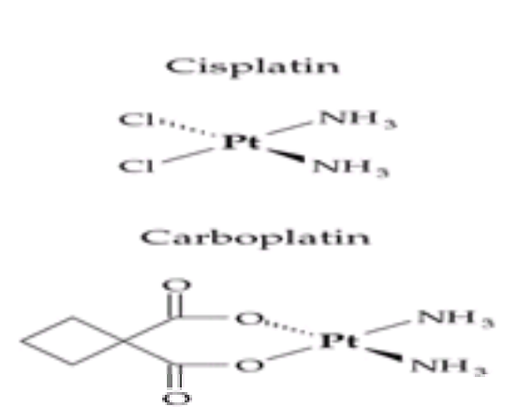



**FIGURE 10-9.** The chemical structures of platinum compounds, cisplatin and carboplatin, which platinate DNA in a manner analogous to alkylation by the nitrogen mustards. Reactive intermediates are formed after spontaneous elimination of chloride (cisplatin) or dicarboxylatecyclobutane (carboplatin).

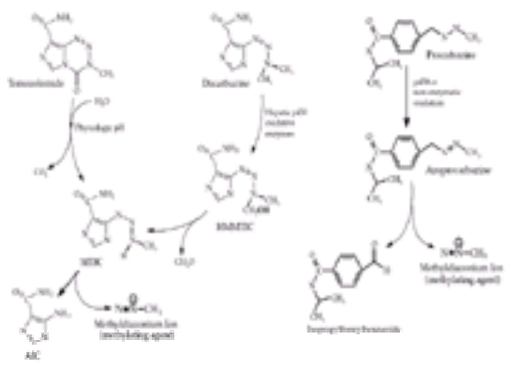


**FIGURE 10-11.** Chemical structures and activation pathways of the methylating agents, dacarbazine, temozolomide, and procarbazine, which are prodrugs. Dacarbazine requires enzymatically catalyzed activation, and temozolomide undergoes spontaneous chemical conversion in solution at physiologic pH to the active metabolite, methyltriazenyl-imidazole carboxamide (MTIC). The metabolic pathway for procarbazine is highly complex and incompletely shown. In addition to the methyldiazonium ion, free radicals can also be generated from azoprocarbazine. AIC, amino-imidazole carboxamide; HMMTIC, hydroxymethyl-MTIC.

## Nitrogen Mustards

The nitrogen mustards were the first class of alkylating agent used to treat cancer and remain the most widely used for childhood cancers. Mechlorethamine (nitrogen mustard), introduced into clinical trials in 1942, was the first drug demonstrated to be effective in the treatment of human cancers. A large number of synthetic nitrogen mustard analogs have since been screened for antitumor activity, and several with greater chemical stability and other pharmacologic advantages have largely supplanted mechlorethamine in clinical practice. Cyclophosphamide and its isomer ifosfamide and melphalan (phenylalanine mustard) are the most widely used in pediatric oncology (Fig. 10-5).

### *Mechlorethamine*

Although the role of mechlorethamine in the treatment of cancer has declined, it is still a model for the chemical reactions of bifunctional alkylators ( Fig. 10-4). The spontaneously formed alkylating intermediate is highly chemically reactive, and it rapidly undergoes hydrolysis, leading to inactivation, or it alkylates a wide variety of molecules, with a propensity to react with the $N^7$ position on guanosine.[179,190,201] Because of this inherent instability, even in aqueous solutions, mechlorethamine must be administered intravenously immediately after preparation to avoid significant loss of activity. Those administering the drug must take precautions, because direct contact with this reactive compound can irritate skin or mucous membranes.

Mechlorethamine has been used primarily in combination with vincristine, prednisone, and procarbazine (MOPP) for the treatment of Hodgkin's disease, but the MOPP regimen is being supplanted as standard therapy for this disease. [202] Topical mechlorethamine has been effective in treating the cutaneous lesions of histiocytosis.[203,204] The use of mechlorethamine as a sclerosing agent in the intracavitary therapy of pleural and pericardial effusions has declined with the advent of less toxic agents such as tetracycline.

The pharmacokinetics of mechlorethamine in humans has not been well delineated. In animals, the drug disappears from plasma in seconds. [190,205] In addition to its rapid spontaneous hydrolysis, mechlorethamine is rapidly metabolized (N-demethylated) in the liver. [205] As a result of this rapid degradation, renal excretion is not likely to play a role in drug clearance.

In addition to its major clinical toxicities of myelosuppression, nausea, and vomiting, mechlorethamine has an anticholinergic effect, leading to diaphoresis, lacrimation, and diarrhea. It is a potent vesicant, producing a sclerosing thrombophlebitis above the site of administration and severe local tissue damage if extravasated. If extravasation occurs, sodium thiosulfate should be injected into the area as rapidly as possible to neutralize the drug. [206] Neurotoxicity in the form of an acute or delayed encephalopathy has been reported with the use of high doses of mechlorethamine. [207]

### *Oxazaphosphorines*

The oxazaphosphorines, cyclophosphamide and ifosfamide, are inactive prodrugs that require biotransformation by hepatic microsomal oxidative enzymes before expressing alkylating activity.[208,209] Cyclophosphamide is a true nitrogen mustard derivative with a bifunctional bischloroethylamine side chain. Ifosfamide is also bifunctional but has one chloroethyl group shifted to a ring nitrogen ( Fig. 10-5). Cyclophosphamide is one of the most widely used anticancer drugs, with a broad range of clinical activity that includes the acute leukemias and a variety of solid tumors ( Table 10-5). It is also used in preparative regimens before bone marrow or peripheral stem cell transplantation and as an immunosuppressant in nonmalignant disorders. Ifosfamide appears to have a similar spectrum of antitumor activity and is undergoing intensive clinical evaluation in the treatment of a variety of childhood cancers. Phase II trials have demonstrated the activity of ifosfamide alone or in combination with etoposide in sarcomas (e.g., Ewing's sarcoma, rhabdomyosarcoma, and osteosarcoma), lymphoma, germ cell tumors, Wilms' tumor, and neuroblastoma.[210,211 and 212]

Cyclophosphamide is usually administered as a single-dose bolus or in fractionated doses over 2 to 3 days. Ifosfamide is administered on a fractionated schedule over 5 days, because in the initial trials, the single-dose schedule produced intolerable nephrotoxicity, cystitis, and neurotoxicity. Ifosfamide has also been administered as a continuous 5-day infusion. The maximally tolerated total dose of ifosfamide is approximately threefold to fourfold higher than an equitoxic dose of cyclophosphamide.[213]

#### Biotransformation

The metabolic pathways of cyclophosphamide and ifosfamide are shown in Figure 10-6. The steps in the biotransformation of these two drugs are qualitatively identical. Hydroxylation of the 4-carbon position on the ring by hepatic microsomal mixed-function oxidases yields the primary 4-hydroxy metabolites, which are in spontaneous equilibrium with the open-ring aldehydes. Hydroxylation of cyclophosphamide is catalyzed primarily by CYP2B6 P450 enzyme with minor contributions from CYP3A4 and CYP2C9, and ifosfamide hydroxylation is catalyzed primarily by CYP3A4 with a minor contribution from CYP2A6.[214] Although not chemically reactive, the 4-hydroxy metabolites are cytotoxic *in vitro* and are thought to be the transport forms of the active alkylating species, phosphoramide mustard and isophosphoramide mustard, which are formed by spontaneous elimination of acrolein from the open-ring aldehydes. Quantitatively, the rate of activation of cyclophosphamide is greater than that of ifosfamide, and this difference in the rate of activation accounts for the difference in clinical pharmacokinetics and maximum tolerated dose of the two isomers.[209,215,216,217,218,219 and 220]

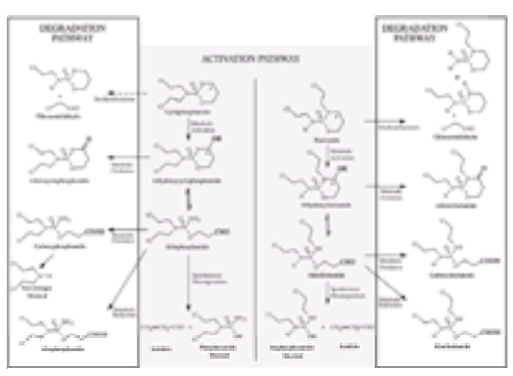


**FIGURE 10-6.** Metabolic pathways for the oxazaphosphorines, cyclophosphamide and ifosfamide. Both compounds must undergo hydroxylation at the 4 position before expressing alkylating activity; this reaction is catalyzed by hepatic microsomal enzymes. The 4-hydroxy metabolites are in spontaneous equilibrium with the open-ring aldehydes (aldophosphamide or aldoifosfamide), which can release acrolein and form the active alkylating mustards (phosphoramide mustard or isophosphoramide mustard). Further oxidation at the 4 position of the primary metabolites leads to the formation of inactive metabolites (ketocyclophosphamide and carboxyphosphamide or ketoifosfamide and carboxyifosfamide), which are excreted in the urine. The open-ring aldehyde metabolites can be chemically reduced to an alcohol (alcophosphamide or alcoifosfamide). Inactivation by dechlorethylation leads to formation of the potentially toxic by-product chloracetaldehyde. This is a minor pathway for cyclophosphamide but is more active with ifosfamide.

Further oxidation of the hydroxyl group at the 4-carbon position on primary metabolites by aldehyde dehydrogenase leads to inactivation. 4-Ketocyclophosphamide and carboxyphosphamide are the principal urinary metabolites of cyclophosphamide. Aldehyde dehydrogenase is found in a wide variety of tissues and in cancer cells.[215,220] The chloroethyl side chain can also be enzymatically cleaved by CYP3A4. Less than 10% of the administered dose of cyclophosphamide is metabolized via this pathway, but up to 50% of the ifosfamide is dechlorethylated, resulting in a greater rate of production of the potentially toxic byproduct chloracetaldehyde compared with cyclophosphamide.[215,216,218,221]

#### Pharmacokinetics

The complexity of oxazaphosphorine metabolism (Fig. 10-6) and the instability of their active metabolites have hampered the study of cyclophosphamide and ifosfamide pharmacokinetics. Methods used to measure plasma drug concentrations have included measurement of total or fractionated radioactivity after administration of radiolabeled cyclophosphamide, total plasma alkylating activity after chemical derivatization, and inactive parent drug or specific metabolites after chromatographic separation.[190,216] The first two assays are nonspecific, because the radioassay cannot differentiate between parent drug and the active and inactive metabolites and because chemical reactivity in the second assay may not correlate with cytotoxicity. Gas chromatographic and high-pressure liquid chromatographic methods are used to measure the concentrations of parent prodrugs and inactive stable metabolites, but these compounds are less relevant because they do not represent the active alkylating species. Chromatographic techniques have also been developed to quantify the active but unstable intermediate metabolites. These

methods use immediate chemical derivatization to prevent breakdown of the metabolites.[209,216,218,220]

The pharmacokinetic behavior of unchanged cyclophosphamide and ifosfamide has been well described. When administrated orally in low doses, 75% to 95% of the cyclophosphamide is absorbed.[209,222,223,224 and 225] The minimal first-pass metabolism after oral administration indicates that the hepatic extraction ratio for cyclophosphamide is low. Plasma concentrations of the active metabolites, 4-hydroxycyclophosphamide and phosphoramide mustard, after oral administration are equivalent to those achieved with intravenous administration.[225] The oral bioavailability of ifosfamide is greater than 95%.[226,227 and 228] Peak concentrations of 4-hydroxy-ifosfamide and chloracetaldehyde were twofold higher than those achieved with the same dose administered intravenously.[209,229]

Cyclophosphamide and ifosfamide are eliminated primarily by hepatic biotransformation to active and inactive metabolites, which are excreted mainly in the urine. Less than 20% of the dose is excreted as unchanged drug in the urine, and biliary excretion of unchanged drug is minimal.[209,223,224,230,231,232,233 and 234] The total body clearance in adults is 30 to 35 mL per minute per $m^2$ and 60 to 80 mL per minute per $m^2$ for cyclophosphamide and ifosfamide, respectively.[219,223,235,236] Total clearance of cyclophosphamide in children (40 to 50 mL per minute per $m^2$) appears to be higher than in adults.[237,238 and 239] The plasma half-life in children (3 to 4 hours) is also reported to be shorter than that in adults (6 to 8 hours).[223,224,230,236,237,238,239 and 240] Ifosfamide clearance in children ranges from 50 to 130 mL per minute per $m^2$, similar to that reported in adults, and the half-life of ifosfamide in children is 1 to 5 hours.[241,242 and 243] Considerable interpatient variability in the disposition and metabolism of the oxazaphosphorines has been observed.[237,241,242,244,245,246 and 247]

Cyclophosphamide and ifosfamide can rapidly induce their own metabolism. With infusional or fractionated dosing, there is a decrease in the plasma half-life and an increase in clearance of the parent prodrugs and an increase in metabolite concentrations.[224,226,240,243,248,249,250,251 and 252] Cyclophosphamide exposure induces the expression of CYP2C9 and CYP3A4 enzyme levels in human hepatocytes.[253] The increase in the rate of metabolism occurs within 12 to 24 hours of the first dose, and a new steady state is achieved by 48 to 72 hours. Over a 5-day course of ifosfamide, the parent drug half-life decreases and the clearance increases by 30% to 50%.[249,251] Conversely, the AUC of the active 4-hydroxy-metabolite increases by 50%,[251,254] which may account for enhanced efficacy of the fractionated dosing schedules.

The oxazaphosphorines are chiral and are administered as a racemic mixture of two optical isomers (i.e., enantiomers) that can be recognized by drug-metabolizing enzymes as being different. Stereochemistry does not appear to have a significant impact on the pharmacokinetics, antitumor activity, or toxicity of cyclophosphamide.[255] However, the clearance of the S-optical isomer of ifosfamide was 1.4-fold more rapid than the clearance of the R-isomer in children, and the half-life of S-ifosfamide was shorter.[243] The clinical significance of this difference in the rate of metabolism of the enantiomers is uncertain.

The fraction of the cyclophosphamide dose that is converted to active metabolites appears to be constant (60% to 70% of the dose), and there is no evidence of saturation of the activating enzymes over a broad dosage range of 100 to 3,000 mg per $m^2$.[230,246] However, at doses of 4,000 mg per $m^2$ used in autologous bone marrow preparative regimens, saturation of drug-activating enzymes becomes apparent.[256,257] Saturation (nonlinearity) of ifosfamide metabolism has also been described at doses exceeding 2,500 mg per $m^2$. The half-life was prolonged to 15 hours, a higher percentage of the drug is excreted in the urine unchanged, and the AUC of ifosfamide metabolites do not increase in proportion to the dose.[218,233,258]

The activated metabolites of cyclophosphamide and ifosfamide appear in plasma rapidly, reach a peak by 2 hours after the dose, and have a half-life of approximately 4 hours.[190,225,259] At equivalent doses, the plasma concentrations of alkylating metabolites of ifosfamide are approximately one-third that generated from cyclophosphamide, presumably because of a difference in the rate of enzymatic activation.[209,215,216,218] Plasma concentrations of the active metabolites are considerably lower than those of the parent prodrug, because of the chemical instability and reactivity of the active 4-hydroxy-metabolites. The plasma concentration of the active 4-hydroxy-metabolites is approximately 1% to 3% of that of the parent drug.[218,254,259,260]

Patients with severe renal function impairment (i.e., creatinine clearance less than 20 mL per minute) have significantly higher plasma alkylating activity as measured by a nonspecific assay.[230,236,261] However, in a single anuric patient, Wagner and associates found no change in the disposition of cyclophosphamide and its activated metabolite,[235] and ifosfamide disposition did not appear to be altered in an anuric child.[262] The degree of cyclophosphamide-related hematologic toxicity does not correlate with the severity of renal insufficiency.[209,263] There is no strong evidence to support dosage modifications of cyclophosphamide in patients with renal dysfunction; however, ifosfamide dosage adjustment may be indicated because of the increased risk of neurotoxicity in patients with renal dysfunction.[109,262] Cyclophosphamide and ifosfamide can be efficiently removed from blood by dialysis.[262,264] The hemodialysis extraction efficiency for 4-hydroxyifosfamide is lower than for the parent drug.[262] Hepatic dysfunction may alter the rate of drug activation and the rate of elimination. With hepatic parenchymal damage, the half-life of cyclophosphamide is prolonged, and peak levels of alkylating activity in plasma are lower.[230]

**Toxicity**

Myelosuppression is the major dose-limiting toxicity of the oxazaphosphorines, but unlike the lipid-soluble alkylating agents, such as the nitrosoureas, it rarely causes cumulative marrow damage. Nausea, vomiting, and alopecia occur in most patients.[54,208]

Hemorrhagic cystitis is a toxicity that is unique to the oxazaphosphorines. It may range from mild dysuria and frequency to severe hemorrhage from bladder epithelial damage. The reported incidence of this complication ranges from 5% to 10% for cyclophosphamide and 20% to 40% for ifosfamide.[54,219] This toxic effect is dose-related and appears to be caused by the activated metabolites and by the biologically active by-products such as acrolein ( Fig. 10-6). The incidence and severity of chemical cystitis can be lessened by aggressive hydration and frequent emptying of the bladder, by bladder irrigation, or by the concurrent administration of mesna (2-mercaptoethane sulfonate). After administration, mesna is rapidly oxidized in plasma to a chemically stable and pharmacologically inert disulfide that is then rapidly excreted by the kidneys and converted back to its chemically reduced active form during tubular transport. It is therefore only active in urine and does not interfere with the antitumor effects of cyclophosphamide or ifosfamide.[117,124,265] Although the dose and schedule of mesna varies, it is commonly administered at a dosage equal to 60% of the total ifosfamide dose, divided into three doses and administered at 0, 4, and 8 hours after ifosfamide.[124] Mesna can be administered orally or intravenously. Mesna also reduces the incidence of oxazaphosphorine-induced bladder cancers in rats, a complication that also has been reported in humans.[219,266]

The oxazaphosphorines are also nephrotoxic. Cyclophosphamide can have a direct renal tubular effect that can result in water retention.[267,268] Ifosfamide produces proximal tubular damage resembling Fanconi's syndrome, with glucosuria, amino aciduria, and phosphaturia. Rickets has been observed in younger children.[269,270,271,272,273,274 and 275] Decreased glomerular filteration rate (GFR) and distal tubular damage manifested by concentrating defects and renal tubular acidosis also have been reported.[276,277] Comprehensive follow-up evaluation of glomerular and tubular function in children previously treated with ifosfamide revealed dysfunction in 78%, including 28% with moderate or severe nephrotoxicity.[278] Cumulative doses exceeding 70 to 80 g per $m^2$ appear to be the primary risk factor.[278,279] Young children are at higher risk for proximal renal tubular damage from ifosfamide.[269]

Other toxic effects of ifosfamide include reversible neurotoxicity characterized by somnolence, disorientation, and lethargy in approximately 20% of patients and, more rarely, hallucinations, coma, and seizures.[280,281] The incidence of neurotoxicity was 50% with oral administration, presumably the result of first-pass metabolism of ifosfamide to neurotoxic metabolites.[218] The neurotoxicity has been attributed to the metabolite chloracetaldehyde ( Fig. 10-6), which results from dechlorethylation of ifosfamide.[282] The dechlorethylation pathway accounts for 50% of ifosfamide metabolism but less than 10% for cyclophosphamide. The incidence of neurotoxicity also appears to be greater in children who previously received high cumulative doses of cisplatin. Cisplatin-induced renal damage might have diminished the rate of elimination of neurotoxic metabolites of ifosfamide in these patients.[283] Neurotoxicity may be reversible or preventable with methylene blue.[284,285] Transient hepatic dysfunction has also been reported with ifosfamide.[219] Cardiac toxicity has been observed in patients treated with high doses (greater than or equal to 100 to 200 mg per kg) of cyclophosphamide. Ifosfamide has also been implicated as a cause of cardiomyopathy and arrhythmias at doses of 10 to 18 g per $m^2$ in a transplant setting.[286]

Although pulmonary toxicity is not commonly associated with the oxazaphosphorines, cases of early- and late-onset interstitial pneumonitis from cyclophosphamide and ifosfamide have been reported.[287,288,289 and 290] Clinical features of drug-induced lung injury typically include fever, cough, dyspnea on exertion, diffuse interstitial infilterates on chest radiographs, and bilateral pleural thickening usually presenting within weeks to months of drug exposure. Factors that appear to augment oxazaphosphorine lung damage include administration of cyclophosphamide in combination with other cytotoxic drugs and the concurrent use of cyclophosphamide and irradiation. Inspired oxygen has also been shown to enhance lung injury in animals.[290] Oxazaphosphorine-induced lung injury appears to be unresponsive to

corticosteroid therapy, and the prognosis is poor.

### Resistance

Mechanisms of resistance to cyclophosphamide involve intracellular inactivation of the activated metabolites and enhanced repair of DNA adducts. [179,208,291] Elevated levels of glutathione resulting from increased activity of the enzyme glutathione-S-transferase, can detoxify the biologically active metabolites of the oxazaphosphorines.[291,292,293,294 and 295] Sensitivity to cyclophosphamide is also inversely correlated with intracellular levels of the enzyme aldehyde dehydrogenase, which oxidizes activated cyclophosphamide metabolites to inactive forms.[195] Intracellular levels of this enzyme can be estimated in tissue or tumor specimens by histochemical staining. Enhanced DNA repair by nucleotide excision repair enzymes or $O^6$-alkylguanine-DNA alkyltransferase may also contribute to resistance. [291]

### Drug Interactions

Compounds known to alter the activity of P450 microsomal enzymes can affect the rate of activation and elimination of the oxazaphosphorines. Phenobarbital pretreatment enhances the rate of metabolism of cyclophosphamide and its activated metabolites in animals and in humans but does not affect the total quantity of alkylating metabolites formed in humans.[143] Cimetidine, which can interfere with the metabolic clearance of many compounds, increases concentrations of alkylating metabolites and prolongs the survival of tumor-bearing mice treated with cyclophosphamide. [296,297] This potential interaction has not been delineated in humans, but the oncologist should be cautious in combining these agents because of the possibility of exaggerated toxicities. Concurrent allopurinol appears to enhance the myelotoxicity of cyclophosphamide.[298] Busulfan, which is administered with cyclophosphamide in transplant preparatory regimens, can block the conversion of cyclophosphamide to its active metabolite, when cyclophosphamide is administered less than 24 hours after a dose of busulfan. [299] Concurrent fluconazole, which can inhibit the metabolism of drugs that are substrates of CYP2C9 and CYP3A4, can also block the activation of cyclophosphamide, whereas dexamethasone and chlorpromazine appear to induce the metabolism of cyclophosphamide.[237]

#### *Melphalan*

Melphalan (L-phenylalanine mustard, Fig. 10-5) is a rationally designed anticancer drug that has the bischloroethylamine moiety attached to the amino acid phenylalanine, with the intention that it would be taken up preferentially by melanin-producing cancers. Although this agent has a broad range of clinical activity in adult cancers (e.g., multiple myeloma, melanoma, breast and ovarian cancers, lymphoma), its use has been limited in the treatment of childhood cancers. At standard doses (35 mg per $m^2$), melphalan appears to be active against rhabdomyosarcoma.[300] The administration of bone marrow ablative doses (140 to 220 mg per $m^2$) of melphalan followed by rescue with autologous bone marrow transplant has resulted in high response rates in children with neuroblastoma, Ewing's sarcoma, and acute leukemia.[185,301,302,303 and 304] Melphalan has also been administered intra-arterially by isolated perfusion for cancers localized to an extremity or the liver. [305,306]

Like other chemically reactive compounds, melphalan is rapidly cleared from the body. It is inactivated after spontaneous hydrolysis or alkylation reactions with plasma or tissue proteins. Melphalan does not appear to undergo any appreciable enzymatic degradation. [54,201,307] The absorption of melphalan after oral administration has been reported to be incomplete and highly variable. [201,307,308,309 and 310] The fraction of a dose absorbed usually ranges from 32% to 100%, but patients with no detectable drug in plasma and urine after an oral dose have been reported. [308,309 and 310] Melphalan bioavailability is higher and less variable when the drug is administered in the fasting state.[311] The incidence of myelosuppression is lower with oral than with intravenous melphalan, and poor therapeutic response may be attributable in part to poor absorption in some patients receiving oral melphalan. [308,312] The disposition of melphalan after intravenous administration in children and adults is similar.[313] With standard parenteral doses, the terminal half-life ranges from 60 to 120 minutes, with a total clearance exceeding 200 mL per minute per $m^2$.[309,310,314,315 and 316] Drug disposition in children and adults is not dose-dependent. Pharmacokinetic parameters in patients receiving high-dose therapy (up to 220 mg per $m^2$) are similar to those found at standard doses.[313,317,318,319,320,321 and 322] Wide interindividual variation in melphalan AUC and clearance has been observed in most studies and has led to the development of pharmacokinetically guided dosing strategies for melphalan. [190]

Renal excretion is a minor route of elimination, accounting for 20% to 30% of total drug clearance. [109,314,323] However, patients with renal dysfunction have a higher incidence of hematologic toxicity, and in nephrectomized animals, the half-life of intravenous melphalan is prolonged, and enhanced myelosuppression is observed.[324,325] In a group of patients with a wide range of renal function, drug clearance after high-dose melphalan was correlated with creatinine clearance, but the decrease in melphalan clearance in patients with renal dysfunction was insignificant compared with the high degree of interindividual variation in drug disposition. [326] In another study of high-dose melphalan, the clearance of melphalan in patients with renal dysfunction (27.5 L per hour) was similar to that in patients with normal renal function (23.6 L per hour).[327]

At standard doses (5 to 35 mg per $m^2$), myelosuppression is the primary toxicity, and cumulative marrow damage has been observed with repeated doses. [54,201] Pulmonary fibrosis and secondary leukemia are late effects associated with the chronic administration of melphalan. [201] At high doses with autologous bone marrow or stem cell reinfusion, gastrointestinal toxicity (e.g., mucositis, esophagitis, or diarrhea) becomes dose limiting. [185,302,321]

### Nitrosoureas

The nitrosoureas are a group of lipid-soluble alkylating agents ( Fig. 10-7) that are highly active in experimental tumor models, including intracranially implanted tumors. The 2-chloroethyl derivatives, carmustine (BCNU) and lomustine (CCNU), are the nitrosoureas most widely used in pediatric oncology. [328,329] Rapid spontaneous chemical decomposition of these compounds in solution generates an alkylating intermediate (chloroethyldiazohydroxide) and an isocyanate moiety that can carbamoylate amine groups on proteins. Alkylation, including crosslinking of DNA by the monofunctional lomustine and the bifunctional carmustine, is generally accepted as the primary mechanism of action of the nitrosoureas.[330,331 and 332] However, the isocyanates can inhibit DNA repair of alkylator damage and may contribute to the antitumor activity and the toxicity of the nitrosoureas.[328,329] The nitrosoureas alkylate the $N^3$ position on cytidine and the $N^7$ and $O^6$ positions on guanosine,[179] but the primary factor determining tumor cell resistance to the nitrosoureas is the capacity to enzymatically repair $O^6$-alkyl-guanosine.[333,334] $O^6$-benzylguanine, an inhibitor of the DNA repair protein, $O^6$-alkylguanine-DNA-alkyltransferase, is being clinically tested in combination with carmustine. [335]

The nitrosoureas have been used primarily to treat patients with brain tumors or lymphomas, and high-dose carmustine has been incorporated into transplant preparative regimens. Delayed and cumulative myelosuppression and other serious long-term cumulative renal and pulmonary toxic effects, which are particularly concerning in children, limit the clinical utility of these agents in combination regimens. [336,337] Carmustine has been incorporated into biodegradable polymer wafers that can be implanted into the tumor cavity after surgical resection for brain tumors. Drug is released slowly from the polymer wafer over 2 weeks, providing prolonged sustained exposure to high concentrations of carmustine locally with a lower risk of systemic toxicity. [338,339 and 340]

#### *Biotransformation and Pharmacokinetics*

In addition to their rapid spontaneous decomposition, nitrosoureas undergo significant hepatic metabolism. [328,341] The cyclohexyl ring of lomustine is hydroxylated at the 4-position to yield two isomeric derivatives that are more soluble and have greater alkylating activity than the parent drug. [328,329] Carmustine is inactivated by denitrosation through the action of microsomal enzymes and glutathione conjugation.[328] As a result of this rapid spontaneous and enzymatic degradation, the clearance of nitrosoureas from plasma is extremely rapid. In early studies of carmustine and lomustine, parent drug could not be detected in plasma after intravenous or oral administration.[342,343] With high-dose carmustine administered by intravenous infusion, the half-life was 22 minutes, and clearance exceeded 2,000 mL per minute per $m^2$.[344] Similar results have been reported with standard doses of the drug (half-life, 22 minutes; clearance, 1,700 mL per minute per $m^2$).[345] The half-life of the active 4-hydroxylated metabolites of lomustine is 3 hours.[346] When administered orally, the nitrosoureas are well absorbed, and lomustine is extensively converted to hydroxylated metabolites presystemically during its first pass through the liver. [347] These results confirm that the metabolites of lomustine are primarily responsible for the drug's antitumor activity. Although carmustine is also well absorbed, severe vomiting after oral administration frequently precludes adequate absorption. [348]

The lipid-soluble nitrosoureas are widely distributed and readily penetrate into the CNS. After equilibration, drug concentrations in the cerebrospinal fluid (CSF) approximate those in plasma, which accounts for the activity of this group of drugs in treating brain tumors. [343,349] Implantation of carmustine-containing polymer wafers into the tumor bed for brain tumors bypasses the blood–brain barrier and provides local drug concentrations that are higher than those achieved with systemic administration. However, the depth of penetration into the brain parenchyma from the wafer is very limited (5 mm at 30 hours) due to the rapid diffusion of drug into

capillaries.[350,351]

### *Toxicity*

Gastrointestinal toxicity (i.e., nausea and vomiting) and cumulative delayed myelosuppression are the most consistent side effects of the nitrosoureas. The nadir of blood counts occurs 4 to 5 weeks after administration, and the platelet count tends to be the most affected. With repeated dosing, chronic marrow hypoplasia develops.[329] With cumulative doses of more than 1,500 mg per $m^2$, progressive renal atrophy has been reported.[352,353] Although in children this complication has been primarily associated with sesmustine (methyl-CCNU), it has also been reported after high cumulative doses of lomustine. Mitchell and Schein recommend that if nitrosourea therapy continues for more than 15 months or if cumulative doses of greater than 1,000 mg per $m^2$ are reached, patients should be evaluated for nephrotoxicity and therapy discontinued if renal size or GFR is significantly decreased.[329] Similar cumulative doses (greater than or equal to 1,500 mg per $m^2$) of carmustine are associated with progressive and frequently fatal pulmonary toxicity characterized by cough, dyspnea, tachypnea, and a restrictive-type ventilatory defect.[290,336,354,355] Carmustine-induced pulmonary toxicity can vary substantially in time to onset, manifestations, outcome, and histopathologic appearance.[336] Long-term follow-up of 17 children with brain tumors treated with carmustine revealed that 6 (35%) had died of pulmonary fibrosis and that all of the surviving patients studied had radiographic abnormalities or restrictive defects on spirometry.[337] Four of the six patients who died presented with pulmonary symptoms 8 to 13 years after treatment. Females appear to be more susceptible to the complication than males.[336,356] Pulmonary fibrosis appears less frequently with lomustine, but cases have been reported.[290] CNS toxicity has been reported rarely.[329] High-dose carmustine (300 to 750 mg per $m^2$) can produce hypotension, tachycardia, flushing, and confusion.[345]

### *Drug Interactions*

In animals, phenobarbital enhances the microsomal metabolism of the nitrosoureas and significantly reduces the antitumor activity of carmustine and, to a lesser extent, that of lomustine.[357] This potential interaction has not been studied in humans. Carmustine, an inhibitor of glutathione reductase, potentiates the hepatotoxicity of high doses of acetaminophen in animals. Liver damage results from the depletion of intrahepatocyte glutathione by a minor but reactive quinone metabolite of acetaminophen.[358]

## Busulfan

The bifunctional alkylating agent, busulfan, is an alkyl alkane sulfonate ( Fig. 10-4). The busulfan alkylation reaction occurs by nucleophilic displacement of the methylsulfonate group on either end of the molecule (Fig. 10-4). Busulfan has a greater propensity to alkylate thiol groups on amino acids and proteins than the nitrogen mustards, but it also can alkylate the $N^7$ position on guanosine.[54]

Busulfan is not water soluble and is commercially available as an oral formulation (2 and 25 mg tablets). An investigational dimethyl sulfoxide (DMSO)-based intravenous solution has been used in pharmacokinetic studies,[359,360] and a microcrystalline suspension (Spartaject Busulfan) is under clinical development for intravenous and intrathecal administration. Busulfan has been used in conventional doses (1.8 mg per $m^2$ per day) as palliative therapy for chronic myelogenous leukemia (CML), and high-dose busulfan (16 mg per kg or 600 mg per $m^2$, in 16 divided doses every 6 hours) has also become an important component of bone marrow transplant preparative regimens, usually in combination with cyclophosphamide.[184]

The pharmacokinetics of busulfan is highly variable and age-dependent.[361,362] Oral busulfan is rapidly absorbed, peaking 1 to 2 hours after the dose; and the bioavailability of oral busulfan in adults and children is 70% (range, 40% to greater than 90%).[359,360,363] Busulfan is a small lipophilic compound and penetrates well across the blood–brain barrier. CSF concentrations at steady state are equivalent to those in plasma.[364,365] The primary route of elimination of busulfan appears to be glutathione conjugation, which is catalyzed by an isoform of glutathione- *S*-transferase (GSTA1-1).[361,366] Busulfan has a short half-life of 2.5 hours and a clearance in children of 80 mL per minute per $m^2$.[359,367] These pharmacokinetic parameters appear to be linear over the wide dosage range used. Compared with adults, busulfan apparent clearance (Cl/F) is more rapid in children, especially children who are 5 years of age or younger[359,368,369]; and the higher apparent clearance in young children is the result of more rapid glutathione conjugation rather than lower bioavailability.[369]

The variability in the disposition of busulfan after oral dosing can result in up to a 20-fold range in systemic drug exposure among patients treated with a fixed dose (16 mg per kg).[361,370] Factors contributing to this variability include the age-dependent clearance, variable bioavailability, hepatic dysfunction, drug interactions, and circadian rhythmicity.[370] The busulfan AUC in young children treated with 1 mg per kg is less than one-half the AUC in adults receiving the same dose ( Fig. 10-8).[361,365,371] On the every-6-hour oral dosing schedule, busulfan trough plasma concentrations exhibited a marked circadian rhythm, with the highest troughs occurring at 6:00 a.m.[362,372]

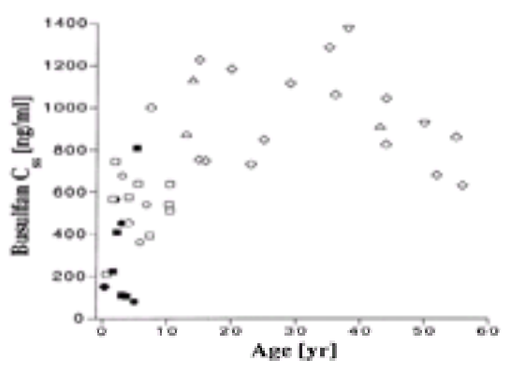



**FIGURE 10-8.** Plasma busulfan steady state concentrations ($C_{ss}$) as a function of age. $C_{ss}$ is derived by dividing the area under the curve by the dosing interval (6 hours). Patients were treated with 16 to 30 mg per kg of busulfan in combination with cyclophosphamide before bone marrow transplant. Closed symbols represent patients who rejected their graft or had a mixed chimera. Patients who experienced grade 0 treatment-related toxicity are designated by the circles, grade 1 toxicity by the squares, grade 2 toxicity by the diamonds, grade 3 toxicity by the upright triangles, and grade 4 toxicity by the inverted triangles. Young children had substantially lower $C_{ss}$, less toxicity, and were at greater risk for graft rejection. (From data presented in Table 1, Table 2, and Table 3 in Slattery JT, Sanders JE, Buckner CD, et al. Graft-rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics. Bone Marrow Transplant 1995;16:31.)

In the transplant setting, busulfan plasma levels (AUC or steady state level, which is derived from AUC and dosing interval) appear to be predictive of hepatic toxicity and graft rejection.[361] In adults, the risk of developing severe hepatic veno-occlusive disease (VOD) is higher when the busulfan AUC exceeds 1,500 µM • minute ($C_{ss}$ of 1,000 ng per mL),[368,373,374] but this toxic level has not been confirmed in children.[375,376] With a busulfan and cyclophosphamide preparative regimen, graft rejection is more common in children (Fig. 10-8) and adults with a busulfan $C_{ss}$ less than 600 ng per mL.[361,368,376] The busulfan AUC or $C_{ss}$ associated with VOD or graft rejection are probably dependent on the preparative regimen administered and the underlying disease.[361] The busulfan dose can be pharmacokinetically adjusted to achieve a $C_{ss}$ or AUC in a safe and effective range by monitoring patients after the first dose.[361,377]

Myelosuppression is the primary toxicity from busulfan. Gastrointestinal toxicity, which is only observed at high doses, includes nausea, vomiting, and mucositis. Busulfan can rarely produce pulmonary toxicity (busulfan lung), which is characterized by diffuse interstitial fibrosis and bronchopulmonary dysplasia. Busulfan lung presents with cough, fever, rales, and dyspnea and usually progresses to respiratory failure.[290,367] Hepatic VOD is observed in up to 40% of patients who are treated with high-dose busulfan without pharmacokinetically guided dosing, and the VOD is severe in 10% of patients.[370,375,378,379] Seizures have also been reported with high-dose therapy, but they are preventable with prophylactic anticonvulsants.[367,380] Girls who receive high-dose busulfan (600 mg per $m^2$) have a high incidence of

severe and persistent ovarian failure. [381]

**Nonclassical Alkylating Agents**

***Platinum Compounds***

Cisplatin and carboplatin are heavy metal coordination complexes (Fig. 10-9) that exert their cytotoxic effects by platination of DNA, a mechanism of action that is analogous to alkylation. Reactive aquated intermediates are formed in solution in a manner similar to the nitrogen mustards (Fig. 10-4). Chloride is the leaving group that is replaced by a water molecule in cisplatin, and dicarboxycyclobutane is the leaving group in carboplatin. These reactive intermediates covalently bind to DNA ($N^7$-position of adenine and guanine) and form intrastrand and interstrand DNA crosslinks. [382,383,384,385 and 386] The rate of reaction of these platinum analogs with water to form reactive intermediates is an important determinant of the stability of the compounds in solution and influences the drugs' pharmacokinetics. [384,387,388] Cisplatin is more reactive than carboplatin and is less stable in aqueous solution. Chloride-containing solutions, such as 0.9% NaCl, are required to stabilize cisplatin before administration.

Cisplatin is an effective agent for the treatment of testicular tumors and has demonstrated activity against osteosarcoma, neuroblastoma, Wilms' tumor, other germ cell tumors, and brain tumors. [389,390,391 and 392] The drug is administered intravenously on a variety of schedules, including a single dose, infused over 4 to 6 hours; divided doses, usually daily for 5 days; and by continuous infusion for up to 5 days. The divided dose and continuous-infusion schedules may lessen the gastrointestinal and renal toxicities. [389] Other strategies used to overcome cisplatin's dose-limiting nephrotoxicity include fluid hydration, mannitol diuresis, the use of hypertonic sodium chloride solutions to promote chloruresis, and the coadministration of sodium thiosulfate or amifostine. [132,393,394 and 395] Cisplatin has been administered regionally in a number of trials, including intraperitoneally for ovarian cancer, intravesicularly for bladder cancer, intrapleurally for the malignant pleural effusions, and intra-arterially for brain tumors and for sarcomas of the extremity, including osteosarcoma. [384,388,396]

Compared with cisplatin in adult trials, carboplatin has a similar spectrum of antitumor activity, but may be less efficacious than cisplatin in several solid tumors, including testicular cancer. [386,397] The pharmacokinetic and toxicity profiles of the two platinum analogs are quite different (Table 10-5 and Table 10-6). [387,398] In children, carboplatin is administered as a bolus dose of 400 to 600 mg per $m^2$ or in divided doses of 400 mg per $m^2$ on 2 consecutive days or 160 mg per $m^2$ daily for 5 days, every 4 weeks. Adaptive dosing formulas, which individualize carboplatin dose based on the GFR, have also been developed for children and are described in Pharmacokinetics below. Carboplatin is active against brain tumors, neuroblastoma, sarcomas, and germ cell tumors. [399,400 and 401]

***Pharmacokinetics***

The chemical stability (reactivity) of the platinum analogs is a critical determinant of their pharmacokinetics. The reactive intermediates of cisplatin and carboplatin are rapidly and covalently bound to plasma protein and tissue. [384,388,402] After binding with plasma or tissue proteins, the reactive platinum intermediates are inactivated. Only the free (unbound) platinum species (including the parent drug) are cytotoxic. [403,404] This interaction of platinum compounds with protein is a time-dependent reaction. For cisplatin, more than 90% of total platinum in plasma is protein bound and inactivated within 2 to 4 hours, [388,405] and this represents the major route of drug elimination. Carboplatin is more chemically stable, and only 20% to 40% of total platinum is protein bound at 2 hours, and this slowly increases to 50% over 24 hours. [406,407 and 408] Tissue-bound platinum may be retained in the body for a prolonged time and is still measurable in plasma for 10 to 20 years after treatment. [409]

Techniques used to assay plasma concentrations of platinum analogs include measurement of total platinum (protein-bound plus unbound forms) by flameless atomic absorption spectrometry or separation of unbound platinum species by ultrafilteration before flameless atomic absorption spectrometry or high-pressure liquid chromatography to separately quantify the parent drug and other unbound (aquated) platinum species.

The pharmacokinetic behavior of bound and unbound, active forms of platinum differ appreciably. For cisplatin, after an initial rapid decay, total platinum (greater than or equal to 95% protein-bound) persists in plasma and can be detected in urine for many days. The terminal half-life of total platinum ranges from 1 to 5 days. [403,405,410] In contrast, the unbound, active platinum species have a much more rapid decline, with a half-life of less than 1 hour, which is primarily a reflection of the chemical reactivity of cisplatin and the avid binding of the reactive intermediates to tissue and plasma protein. [403,411] In children receiving cisplatin, the half-lives of total and ultrafilterable (unbound) platinum are 44 hours and 40 minutes to 1.5 hours, respectively. [392,412,413]

Approximately 50% of the platinum administered as cisplatin is excreted in the urine over 4 to 5 days, most in an inactive form. [405,414,415] Initially, total platinum clearance equals or exceeds creatinine clearance, reflecting excretion of unbound platinum species, but as protein binding becomes extensive, renal clearance of total platinum drops to only a small fraction of creatinine clearance. [403] The renal clearance of the unbound, ultrafilterable species of platinum can actually exceed creatinine clearance, suggesting tubular secretion. [412,416,417] In children, the clearance of cisplatin was not related to the GFR. [412] Approximately 25% of unbound platinum species is excreted in the urine, and the degree of renal excretion is schedule dependent (i.e., greater with short infusions). [418] In patients with impaired renal function, the peak level of active, unbound platinum was elevated, but the terminal half-life was not prolonged, presumably because of the rapid reaction of these active species with plasma and tissue protein leading to inactivation. [414,419] However, dosage reductions in patients with renal dysfunction may be indicated because of the drug's nephrotoxic effects, which could further impair renal function. [108,388]

The disposition of carboplatin is characterized by a lower rate and degree of protein binding than for cisplatin. As a result, the terminal half-life of unbound carboplatin is longer (2 to 3 hours), and renal excretion is the primary route of elimination. [386,387 and 388,406,408,413,415,420] By 24 hours, as much as 70% of the total platinum from carboplatin is excreted in the urine, most as parent drug. Carboplatin is dialyzable in patients with severe renal insufficiency. [421,422]

Pharmacokinetic parameters for carboplatin in children are similar to those in adults. The total clearance in children with a normal creatinine clearance is approximately 70 mL per minute per $m^2$, and the half-life is 2 to 3 hours. [413,423,424 and 425] In children younger than 5 years of age, carboplatin clearance was 120 mL per minute per $m^2$, but in children younger than 1 year of age, the clearance was 75 mL per minute per $m^2$. [426] These age-related differences in carboplatin clearance appear to be related to differences in the GFR. The variability in carboplatin clearance supports the use of the adaptive dosing formulas based on GFR described subsequently.

The total clearance of ultrafilterable carboplatin is highly correlated with GFR (Fig. 10-10), [105,387,406,408,425,426 and 427] and patients with renal dysfunction and higher carboplatin AUCs have a greater probability of experiencing dose-limiting hematologic toxicity. These associations allowed the development of adaptive dosing formulas for individualizing carboplatin dose based on GFR in adults and children (Table 10-7). [105,423,425,427,428,429 and 430] The use of these formulas to calculate an individualized dose decreases the variability in systemic drug exposure (AUC) and reduces the incidence of severe thrombocytopenia. [406,431,432] When administered as a single dose in combination with ifosfamide and etoposide, a targeted carboplatin AUC of up to 10 mg • minute per mL was tolerable. [431] In ovarian and testicular cancers in adults, a carboplatin AUC of 5 to 7 mg • minute per mL was associated with a higher response rate and a lower risk of disease recurrence. [427,432]

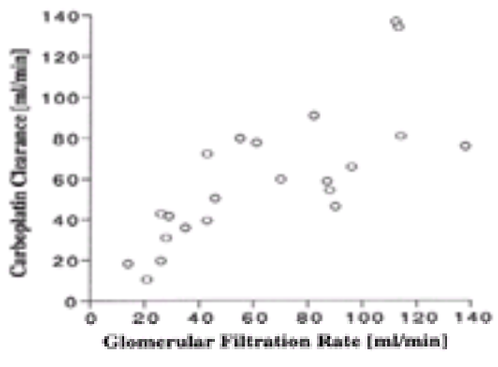



**FIGURE 10-10.** Relation between carboplatin clearance and glomerular filteration rate as measured by $^{51}$Cr-EDTA clearance in 22 children. (Adapted from Newell

DR, Pearson ADJ, Balmanno K, et al. Carboplatin pharmacokinetics in children: the development of a pediatric dosing formula. J Clin Oncol 1993;11:2314.)

| Population | Formula[a] |
|---|---|
| Adults[418] | $D\ [mg/m^2] = 0.015 \times CL_{cr}[mL/min/m^2] \times \left(\frac{prePlt - trgtPlt}{prePlt} \times 100 - priorRx\right) + 86$ |
| Adults[419] | $D\ [mg] = trgtAUC\ [mg \cdot min/mL] \times (GFR\ [mL/min] + 25)$ |
| Children[419] | $D\ [mg/m^2] = trgtAUC\ [mg \cdot min/mL] \times (0.93 \times GFR\ [mL/min/m^2] + 15)$ |
| Children[419] | $D\ [mg] = trgtAUC\ [mg \cdot min/mL] \times GFR\ [mL/min] + (0.36 \times BW\ [kg])$ |

BW, body weight; $CL_{cr}$, creatinine clearance; D, dose; GFR, glomerular filteration rate estimated by radioisotopic method; prePlt, pretreatment platelet count; priorRx, 0 for previously untreated and 17 for previously treated; trgtAUC, target systemic drug exposure (AUC); trgtPlt, target nadir platelet count.
[a]Units for each parameter are listed in the brackets.

**TABLE 10-7. ADAPTIVE DOSING FORMULAS FOR TARGETING CARBOPLATIN DOSE TO ACHIEVE A DESIRED NADIR PLATELET COUNT OR AREA UNDER THE CURVE (AUC), WITH THE TARGET AUC RANGING FROM 7 TO 10 MG•MINUTE PER ML**

***Toxicity***

The toxicity profiles of these two platinum analogs are strikingly different. Cisplatin is associated with only mild myelosuppression but produces significant and potentially irreversible nephrotoxicity, ototoxicity, and neurotoxicity; the dose-limiting toxicity of carboplatin is hematologic toxicity, primarily thrombocytopenia, and the nonhematologic toxicities observed with cisplatin are only seen at doses of carboplatin exceeding 800 mg per $m^2$.[384,387,398,433]

Nephrotoxicity, manifested as azotemia and electrolyte disturbances (especially hypomagnesemia requiring oral supplementation), was the dose-limiting toxicity in the initial clinical trials with cisplatin.[433,434 and 435] The exact mechanism of cisplatin nephrotoxicity is not defined, but patients experience a reduction in renal blood flow and GFR and a loss of tubular function. Pathologic changes are seen primarily in the renal proximal and distal tubule epithelium and collecting ducts.[394,433,436,437] Renal damage from cisplatin is cumulative.

Although pretreatment hydration, diuresis, chloruresis, and less-toxic dose schedules have reduced the incidence and severity of cisplatin-induced nephrotoxicity, moderate and permanent reductions in the GFR of patients receiving cisplatin have been documented.[438,439 and 440] However, in a long-term follow-up study of 40 children who received a median of 500 mg per $m^2$ of cisplatin, 22 of the 24 patients with abnormally low end-therapy GFRs partially recovered, with a median increase in GFR of 13 mL per minute per $m^2$.[441] The long-term nephrotoxic effects of cisplatin in infants are similar to those reported in older children.[442]

Pretreatment with amifostine lessens the severity of renal damage by cisplatin without altering the antitumor effect of the drug in adults.[132,443] The proportion of patients who experienced a greater than or equal to 40% reduction in creatinine clearance after at least four courses of cisplatin was 32% with cisplatin alone and 10% when amifostine was administered before cisplatin.[444] Amifostine also lowered the incidence and severity of neurotoxicity and ototoxicity in adults.[443]

As a result of its nephrotoxic effects, cisplatin can alter its own elimination rate and that of other drugs, such as methotrexate, that rely on renal excretion.[445] In one series, the renal clearance of ultrafilterable platinum fell from almost 500 mL per minute with the first course to 150 mL per minute by the fourth course in patients receiving repeated doses, probably as a result of decreased renal tubular secretion of the drug.[446] Higher renal cortical concentrations of platinum were found at autopsy in patients who had clinical renal toxicity than in patients without evidence of renal toxicity.[447]

As methods to prevent nephrotoxicity have allowed the administration of higher single and cumulative doses of the drug, ototoxicity and peripheral neuropathy have become more prominent.[433] Cisplatin causes a reversible sensory peripheral neuropathy (i.e., numbness, tingling, and paresthesias) at cumulative doses of 300 to 600 mg per $m^2$.[281,433] Lhermitte's sign (an electric shock sensation when the neck is flexed) is common at high cumulative doses of cisplatin.[448] Symptoms may progress after discontinuation of cisplatin and persist for months to years. Seizures and encephalopathy have also been reported in children receiving intensive cisplatin therapy.[449] The irreversible hearing loss is in the high-frequency range and appears to be related to a cumulative dose of cisplatin of greater than 400 mg per $m^2$.[401,450,451 and 452] Other toxic effects include prominent nausea and vomiting, mild myelosuppression, Raynaud's phenomenon, and hypersensitivity reactions.[389]

Carboplatin's myelosuppressive effects are delayed, with platelet nadirs typically seen up to 3 weeks after the dose and milder granulocyte nadirs at 3 to 4 weeks, which determines the frequency with which the drug can be administered. Some patients require 5 to 6 weeks to recover completely.[453] Not only are the nephrotoxicity, ototoxicity, and peripheral neuropathy from carboplatin milder than cisplatin, but the nausea and vomiting, which can be dose-limiting with cisplatin, are also less severe.[387,453,454] High cumulative doses of carboplatin are associated with a small drop in GFR and serum magnesium, but these changes are usually not clinically significant.[455] Hypersensitivity reactions to carboplatin are relatively common, and the risk increases after multiple cycles of therapy.[456,457]

***Resistance***

Studies in preclinical tumor models have implicated several possible mechanisms of resistance to platinum compounds.[382,458,459] Decreased drug accumulation may be related to altered drug uptake or the presence of a membrane efflux pump. Increased intracellular levels of thiol-containing compounds, such as glutathione and metallothionein, can react with and inactivate the active aquated forms of cisplatin and carboplatin. The enhanced repair of platinum-DNA adducts by the nucleotide excision repair pathway removes the cytotoxic lesion produced by the platinum analogs. Platinum-induced DNA damage activates apoptosis and expression of cellular proteins that suppress the apoptotic response to this damage or loss of mismatch repair activity may alter sensitivity to the platinum analogs.[178,460]

**Dacarbazine**

Although dacarbazine (Fig. 10-11) was originally developed as an inhibitor of purine biosynthesis, it does not exert its antitumor effects as an antimetabolite.[461] Dacarbazine is a prodrug that undergoes hepatic microsomal metabolic activation (N-demethylation), which is catalyzed primarily by CYP1A2, to the active metabolite, methyltriazenyl-imidazole carboxamide (MTIC).[462] MTIC then spontaneously decomposes into a reactive methylating species (methyldiazonium ion) and the primary circulating metabolite aminoimidazole carboxamide (AIC).[461,463,464] The methyldiazonium ion can methylate nucleophilic sites, including the $O^6$ and $N^7$ positions on guanosine, but it cannot form crosslinks.

Dacarbazine is generally administered intravenously (150 to 250 mg per $m^2$) on a divided once-daily dosage schedule for 5 days. It is used primarily in the treatment of sarcomas, neuroblastoma, and Hodgkin's disease. Absorption after oral administration is slow, incomplete, and variable.[465] After intravenous administration, the drug is rapidly cleared from the plasma, with a terminal half-life of 40 minutes and a total clearance of 450 mL per minute per $m^2$. One-half of the dose is excreted unchanged in the urine, and renal clearance exceeds the GFR, suggesting the drug is also eliminated by renal tubular secretion.[466] The remainder of the dose presumably undergoes biotransformation. The half-life and renal clearance of the metabolite AIC are similar to that of the parent drug.[466] Methylated DNA adducts in white blood cells of patients treated with dacarbazine (250 to 800 mg per $m^2$) increase rapidly during the first hour after treatment but then decline with a more prolonged half-life (72 hours) than the parent drug.[467]

When dacarbazine was administered as a 1,000 mg per $m^2$ infusion over 24 hours, the steady-state plasma concentration was 8.6 mg per mL.[468] Other pharmacokinetic parameters derived from the study of this schedule included a total clearance of 110 mL per minute per $m^2$, a volume of distribution at steady state of 23 L per $m^2$, and a terminal half-life after infusion of 3 hours.

Gastrointestinal toxicity, consisting of moderate to severe nausea and vomiting, is the primary toxicity and is frequently dose-limiting. Tolerance usually develops over the 5-day course of administration. At standard doses, myelosuppression is mild. Other side effects include a flu-like syndrome with malaise, fever, and myalgias; mild hepatic dysfunction; and local pain at the site of intravenous injection. Rare cases of liver failure and death from VOD and hepatic vein thrombosis (Budd-Chiari syndrome) have been associated with the use of this drug.[469]

**Temozolomide**

The methylating agent temozolomide is structurally and mechanistically related to dacarbazine. Like dacarbazine, temozolomide is a prodrug, but temozolomide does not require enzymatic activation in the liver. In solution at physiologic pH, temozolomide spontaneously decomposes to MTIC, the same active metabolite that is derived by enzymatic N-demethylation of dacarbazine (Fig. 10-11).[470,471]

Temozolomide is only available in capsules (5, 20, 100, and 250 mg sizes) for oral administration. It is insoluble in aqueous solution, and this has precluded the development of an intravenous formulation. Based on preclinical studies [470,472] that demonstrated divided dosing schedules had greater antitumor effect than a single bolus dose and on the initial phase I clinical trial, [473] in which responses were only observed on the divided dose schedule, temozolomide is administered as a single daily dose for 5 consecutive days. The recommended dose for children is 200 mg per $m^2$ per day (1,000 mg per $m^2$ per course).[474,475] A continuous daily dosing schedule is also being investigated, and a dose of 75 mg per $m^2$ per day appears to be tolerable for 6 to 7 weeks in adults.[476] Temozolomide is used in children exclusively for the treatment of recurrent brain tumors, but it also has activity against sarcomas in preclinical models.[470]

Absorption of temozolomide from the gastrointestinal tract is rapid and complete.[473,477] The peak concentration of temozolomide is achieved in plasma within 1.5 hours of the dose.[475] When administered with food, the bioavailability is slightly lower but remains greater than 90%.[478] Temolozomide is also rapidly eliminated. Its half-life (1.8 hours) is similar to the drug's half-life in a pH 7.4 phosphate buffer solution *in vitro*,[471] suggesting that decomposition to the active metabolite, MTIC, is the primary route of elimination for temozolomide. A pharmacokinetic study of radiolabeled temozolomide confirmed that AIC, which is the end-product of temozolomide decomposition to MTIC, is the primary urinary metabolite.[479] In children, 5% to 15% of the dose of temozolomide was recovered in urine as unchanged drug.[475] The clearance of temozolomide is 100 mL per minute per $m^2$. The active metabolite, MTIC, is much less stable and has an estimated half-life of 2.5 minutes and clearance exceeding 5,000 mL per minute per $m^2$.[479,480] Temozolomide is widely distributed in tissues and penetrates well across the blood–brain barrier,[477] and could therefore be considered the transport form for MTIC.

Myelosuppression is the dose-limiting toxicity of temozolomide. Nadir neutrophil and platelet counts typically occur 21 days after the start of therapy, and recovery of blood counts may take 7 to 10 days.[471,474,475] This delayed myelosuppression necessitates administering temozolomide on a 28-day schedule. The myelosuppression from temozolomide does not appear to be cumulative.[473] Nonhematologic toxicities are mild and include nausea and vomiting, which can be controlled by pretreatment with standard antiemetics, headache, fatigue, constipation, and serum transaminase elevations.[477]

The DNA repair protein $O^6$-alkylguanine-DNA alkyltransferase removes the methyl adduct from the $O^6$-position of guanine. Although this adduct accounts for only 5% of DNA adducts formed by temozolomide,[470] it is thought to be the primary cytotoxic lesion. Tumor cell lines with high levels of this repair protein are resistant to the cytotoxic effect of temozolomide.[470,471,481] Depletion of $O^6$-alkylguanine-DNA alkyltransferase with the modulating agent $O^6$-benzylguanine, markedly enhances the cytotoxic effects of temozolomide.[482] Loss of DNA mismatch repair capacity enhances to resistance to temozolomide.

**Procarbazine**

Procarbazine is a methylhydrazine analog that was originally synthesized as monoamine oxidase inhibitor but was discovered to have antitumor activity in animals. Procarbazine is currently used as part of the MOPP chemotherapy regimen for the treatment of Hodgkin's disease[483] and is also active against brain tumors.[484] Procarbazine is a prodrug that requires metabolic activation *in vivo* to express its antitumor activity.[461] This activation yields methylating and free radical intermediates, which appear to produce the drug's antitumor effect.

The spontaneous chemical decomposition and biotransformation of procarbazine is complex and beyond the scope of this chapter, but it has been reviewed elsewhere.[461,464,485] Metabolic activation probably occurs in the liver and is catalyzed by the cytochrome P-450 enzyme complex ( Fig. 10-11).[486] In liver perfusion studies, procarbazine is extensively converted to its active azo-metabolite.[487]

The disposition of procarbazine and its active intermediates has not been well characterized in humans. The drug is rapidly and completely absorbed from the gastrointestinal tract,[488] and it undergoes complete first-pass conversion to cytotoxic metabolites, which probably accounts for the activity of the drug when administered orally. After intravenous administration, procarbazine is rapidly metabolized and has a half-life of less than 10 minutes.[489] The metabolites of procarbazine are excreted primarily in the urine. Procarbazine or unidentified metabolites enter the CSF readily.[489] Drugs such as phenobarbital and phenytoin that are capable of inducing hepatic microsomal enzymes can increase the rate of procarbazine activation.[461] Procarbazine can inhibit the biotransformation of the barbiturates, phenothiazines, and other sedatives, resulting in potentiation of their sedative effects. The inhibition of monoamine oxidase by procarbazine can put patients at risk for hypertensive reactions from foods high in tyramine (e.g., bananas, wine, and cheese). Procarbazine also appears to alter its own metabolism over a 14-day course of therapy. The plasma concentrations of procarbazine metabolites differ markedly between day 1 and 14 of treatment.[490]

The primary toxicities of procarbazine include nausea, vomiting, and myelosuppression. Some patients develop evidence of neurotoxicity consisting of paresthesias, somnolence, depression, or agitation. Neurotoxicity is prominent with high-dose intravenous administration.[491] Patients are also at risk for the long-term toxicities, including azoospermia, ovarian failure, and teratogenic and carcinogenic effects.[461]

## ANTIMETABOLITES

The antimetabolites are structural analogs of vital cofactors or intermediates in the biosynthetic pathways of DNA and RNA. By acting as fraudulent substrates for the enzymes in these pathways, antimetabolites inhibit synthesis of the nucleic acids and their building blocks or are incorporated into the DNA or RNA, resulting in a defective product. Antimetabolites that are used in the treatment of childhood cancers include the folate analog methotrexate, the purine analogs mercaptopurine and thioguanine, and the pyrimidine analogs cytarabine and fluorouracil. The structures of these antimetabolites and their naturally occurring counterparts are shown in Figure 10-12.

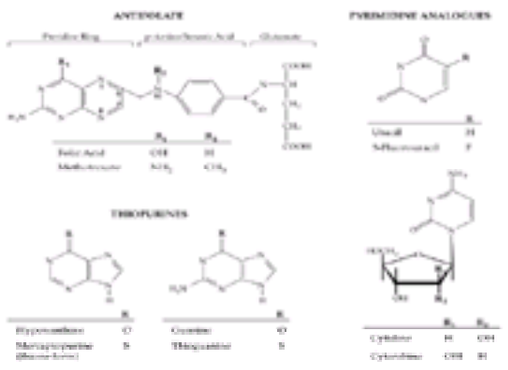



**FIGURE 10-12.** Chemical structures of commonly used antimetabolites compared with the structures of corresponding endogenous compounds of which they are analogs.

In general, the clinical pharmacology of these agents is similar to that of the endogenous compounds that they structurally resemble. The absorptive, metabolic, and excretory pathways are frequently shared by the endogenous compound and the antimetabolite. The rate of elimination of the antimetabolites is usually rapid. Most of the antimetabolites are prodrugs that require metabolic activation within the target cell to express their cytotoxic effects. The purine and pyrimidine analogs, for example, require intracellular conversion to phosphorylated nucleotides, which are the active forms of these drugs. Because most antimetabolites interfere directly with DNA synthesis, they are cell-cycle and S-phase specific; the maximum cytotoxic effect occurs in cells that are synthesizing DNA. This partially explains the schedule dependence of this class of anticancer drugs. More prolonged drug exposure that results from administering these agents by continuous infusion or by chronic daily dosing increases the chance of exposing a higher proportion of the tumor cell population to the drugs during active DNA replication.

**Methotrexate**

Methotrexate is the most widely used antimetabolite in childhood cancers. It is effective in the treatment of ALL, non-Hodgkin's lymphoma, the histiocytoses, and osteosarcoma. Methotrexate is administered on an intermittent schedule by variety of routes, including oral, intramuscular, subcutaneous, intrathecal, and intravenous. Chronic oral or intramuscular therapy is administered weekly at a dose of 20 mg per $m^2$. With intravenous therapy, an extraordinarily wide range of doses has been used, ranging from a 10 mg bolus to 33,000 mg per $m^2$ as a 24-hour infusion. Doses above 100 to 300 mg per $m^2$, which are usually administered by continuous infusion, must be followed by a course of the rescue agent leucovorin (5-formyl-tetrahydrofolate) to prevent the development of severe toxicities.

The loading and infusion doses required to achieve a desired steady-state plasma concentration ($\{MTX\}_{plasma}$) can be estimated from the following formulas[492]:

Loading dose (mg per $m^2$) = 15 • $\{MTX\}_{plasma}$ (μM)

Infusion dose (mg per $m^2$ per hour) = 3 • $\{MTX\}_{plasma}$ (μM)

For example, to achieve a steady state plasma concentration of 10 μM, the loading dose would be 150 mg per $m^2$, followed by an infusion of 30 mg per $m^2$ per hour. Infusion durations of up to 42 hours are tolerable when followed by leucovorin rescue. In clinical practice, infusion durations range from 4 to 36 hours depending on the type of cancer being treated. Patients who are treated with a high-dose methotrexate infusion must be adequately hydrated and alkalinized to prevent precipitation of methotrexate in acidic urine, and routine monitoring of serum creatinine and plasma methotrexate concentrations is mandatory to determine the duration of leucovorin rescue. For most infusion regimens, 12 to 15 mg per $m^2$ of leucovorin should be continued every 6 hours until plasma methotrexate concentration falls to 0.05 to 0.10 μM.

***Mechanism of Action***

Methotrexate is a structural analog of folic acid, a required cofactor for the synthesis of purines and thymidine. As a result of the substitution of an amino group for the hydroxyl group at the 4-position on the pteridine ring of folic acid ( Fig. 10-12), methotrexate is a tight-binding inhibitor of DHFR, the enzyme responsible for converting folates to their active, chemically reduced (tetrahydrofolate) form.[493,494] 10-Formyltetrahydrofolate acts as the single carbon donor in the de novo purine synthetic pathway, and 5,10-methylenetetrahydrofolate donates its single-carbon group and is oxidized to dihydrofolate in the conversion of deoxyuridylate (dUMP) to thymidylate by thymidylate synthase. In the presence of an excess of methotrexate, intracellular tetrahydrofolate pools are depleted, leading to depletion of purines and thymidylate and inhibition of DNA synthesis. Accumulation of partially oxidized dihydrofolic acid, resulting from the inhibition of DHFR, appears to contribute to the inhibition of de novo purine synthesis.[494,495] and [496] A critical determinant of methotrexate cytotoxicity is the rate of thymidylate synthesis, because the synthesis of thymidylate from uridylate is the only reaction that oxidizes the tetrahydrofolate cofactor to the inactive dihydrofolate form. Another determinant is achieving an intracellular methotrexate concentration that is in excess of DHFR binding sites, because intracellular levels of this target enzyme are 20-fold to 30-fold higher than required to maintain tetrahydrofolate pools.[493,497,498]

Methotrexate shares membrane transport processes and intracellular metabolic pathways with the naturally occurring folates. It competes with the tetrahydrofolates for an energy-dependent transport system for cell entry. On entry, methotrexate is rapidly and tightly bound to DHFR, and uptake into the target cell is essentially unidirectional until the enzyme binding sites are saturated, allowing for even greater intracellular accumulation of drug.[493]

With the accumulation of free intracellular drug in excess of DHFR binding sites, methotrexate, like the naturally occurring folates, is metabolized intracellularly to polyglutamated derivatives, which cannot readily efflux from the cell. Methotrexate polyglutamate formation enhances the cytotoxicity of the drug by allowing greater accumulation of free intracellular drug and retention of the drug within the cell, even after extracellular drug is cleared. Methotrexate polyglutamates are also more potent inhibitors of DHFR and are capable of directly inhibiting other enzymes in the synthetic pathways for thymidine (thymidylate synthase) and purines.[494,497,499,500] Methotrexate polyglutamate formation is optimal *in vitro* when cells are exposed to high concentrations for prolonged periods, and children with ALL randomized to receive high-dose methotrexate as initial induction therapy had higher methotrexate polyglutamate levels in their lymphoblasts than patients randomized to low-dose methotrexate.[501,502] The lymphoblasts from children with ALL and good prognostic features, such as B-lineage immunophenotype, hyperdiploidy, young age, low presenting white blood cell count, and female sex, tend to accumulate methotrexate polyglutamates more efficiently than blasts from higher risk patients, suggesting that ALL in lower risk patients may be more sensitive to the antileukemic effects of methotrexate.[502,503,504] and [505]

***Pharmacokinetics***

At standard oral doses of 7.5 to 20.0 mg per $m^2$, the rate and extent of absorption of methotrexate is highly variable ( Fig. 10-13).[81,506,507,508] and [509] Peak plasma concentrations can occur from 0.5 to 5.0 hours after oral administration, and the percentage of the dose that is absorbed ranges from 5% to 97%.[506] The AUC of oral methotrexate ranged from 0.63 to 12 μM • hour at a dose of 18 to 22 mg per $m^2$, and over a broader dosage range, the AUC correlated poorly with the dose ( Fig. 10-13).[81] In patients who are studied after multiple doses, there was also considerable intrapatient variation in the AUC.[81] Absorption of methotrexate is saturable, and as the dose is increased, the fraction of the dose that is absorbed diminishes.[510,511,512] and [513] Simply increasing the dose in patients who have low plasma concentrations after standard oral doses may not overcome poor bioavailability. The bioavailability of oral methotrexate can also be significantly reduced when administered with food.[514] Despite this variability with oral dosing, there was no relation between the relapse rate and methotrexate pharmacokinetic parameters, such as peak concentration, AUC, and erythrocyte methotrexate levels.[81,515,516] When administered intramuscularly or subcutaneously, methotrexate is completely absorbed.[512,513,517,518]

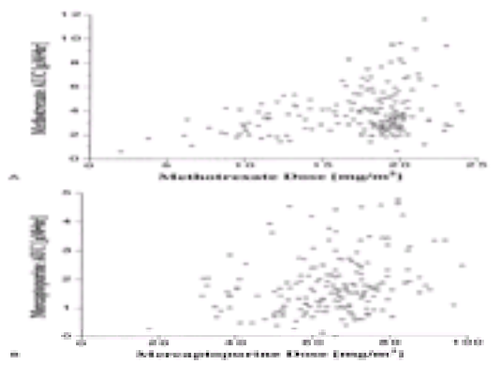


**FIGURE 10-13.** Area under the plasma concentration-time curve (AUC) as a function of dose in children with acute lymphoblastic leukemia that is treated with weekly oral methotrexate **(A)** and daily oral mercaptopurine **(B)**. Drug exposure was highly variable and did not correlate with the dose for both agents. (From Balis FM, Holcenberg JL, Poplack DG, et al. Pharmacokinetics and pharmacodynamics of oral methotrexate and mercaptopurine in children with lower risk acute lymphoblastic leukemia: a joint Children's Cancer Group and Pediatric Oncology Branch study. Blood 1998;92:3569.)

The disposition of methotrexate in children differs from that in adults.[519,520,521 and 522] In one study, children had lower plasma concentrations of methotrexate and excreted the drug in the urine more rapidly after a 6-hour infusion than did adults.[523] The volume of distribution was also greater in children. Within the pediatric age group, the clearance of methotrexate (normalized to body surface area) is also age-dependent.[524] Children younger than 10 years of age (n = 94) had a clearance of 160 mL per minute per $m^2$, compared with 110 mL per minute per $m^2$ in those older than 10 (n = 21). Infants (younger than 1 year old) may have a slightly lower (15%) clearance rate than children, but dose adjustments do not appear to be necessary, because the degree of toxicity is similar and 42-hour methotrexate plasma concentrations after high-dose infusions, are similar to those in older children.[111]

The plasma disappearance of methotrexate is multiphasic, with a terminal half-life of 8 to 12 hours.[492,494,525] Retention of the drug in large extravascular fluid collections, such as ascites or pleural fluid, is associated with prolongation of the half-life as a result of slow release of retained drug into the circulation.[494,525] This prolonged exposure to the drug can increase the risk for toxicity. Patients who have large extravascular fluid collections and are receiving methotrexate should have their methotrexate levels monitored closely.

Methotrexate is eliminated primarily by renal excretion, undergoing glomerular filteration and renal tubular reabsorption and secretion.[526,527] Approximately 70% to 90% of a dose is excreted unchanged in the urine, most within the first 6 hours. The renal clearance of methotrexate can exceed the rate of creatinine clearance. In patients with significant renal dysfunction, methotrexate clearance is delayed, resulting in prolonged drug exposure and a greater risk of severe toxicities. High-dose methotrexate should not be given to patients with a creatinine clearance of less than 50% to 75% of normal. Low-dose therapy should be withheld in patients with a serum creatinine level greater than 2 mg per dL. Any patient who is suspected of having renal dysfunction and who receives methotrexate should have the plasma levels closely monitored and receive leucovorin if drug clearance is delayed.[528]

Methotrexate is also metabolized in the liver to 7-hydroxy-methotrexate.[529] Although this is a minor route of elimination, plasma concentrations of 7-hydroxy-methotrexate can be equivalent to or exceed those of methotrexate after high-dose infusions, because of the slower clearance of the metabolite.[525,530,531 and 532] 7-Hydroxy-methotrexate may compromise the cytotoxicity of methotrexate by competing for membrane transport and polyglutamation. Once polyglutamated, however, 7-hydroxy-methotrexate appears to be able to bind to and inhibit DHFR.[493] Methotrexate clearance is not significantly altered with hepatic dysfunction, but modification of the methotrexate dose in patients with abnormal liver function tests may be indicated to avoid additional hepatic damage.

Total renal and metabolic methotrexate clearance is approximately 100 mL per minute per $m^2$, but it may vary widely among patients.[494,532,533] In patients with normal creatinine clearance, there is not a good correlation between methotrexate clearance and creatinine clearance.[533] Renal tubular dysfunction, which is not measured by creatinine clearance, may account for this disparity. A small test dose of methotrexate can accurately predict the kinetics and steady-state concentration of a high-dose infusion.[534] Optimal management dictates that each course of high-dose methotrexate be closely monitored by following renal function and plasma methotrexate concentration to determine the dose and duration of leucovorin rescue.

Penetration of systemically administered methotrexate into CSF is only 3% in patients without meningeal tumor spread,[535,536] but is 20% in patients with leptomeningeal carcinomatosis.[536] At infusion rates exceeding 3,500 mg per $m^2$ over 24 hours, the CSF methotrexate concentration is typically greater than 1 µM[536]; and high-dose methotrexate infusion regimens are effective for treating and preventing leptomeningeal leukemia.[537]

***Toxicity***

The primary toxic effects of methotrexate are myelosuppression and orointestinal mucositis, which occur 5 to 14 days after the dose. The development of toxic reactions is related to the concentration of drug and the duration of exposure.[492,494,525] In patients receiving a 6-hour infusion of methotrexate, a 48-hour methotrexate concentration above 1 µM was associated with the development of significant toxicity.[525] These toxicities can be prevented by administration of leucovorin. With the use of therapeutic drug monitoring and continuation of leucovorin rescue until plasma methotrexate concentration has fallen below 0.05 to 0.10 µM, the toxicity of high-dose methotrexate can be avoided in most patients.[492,494]

Nephrotoxicity observed with high-dose methotrexate can delay methotrexate clearance and markedly intensify the drug's other toxic effects.[538,539] The renal damage may be related to precipitation of methotrexate or 7-hydroxy-methotrexate in acidic urine or to direct toxic effects on the renal tubule.[494,539] Drug precipitation can be prevented by vigorous intravenous hydration and alkalinization of the urine. In one study, aggressive hydration and alkalinization resulted in enhanced excretion of methotrexate (lower plasma drug concentrations) and a decreased incidence of severe methotrexate toxicity.[540]

The development of renal dysfunction during high-dose methotrexate is a medical emergency. Patients must be closely monitored and the leucovorin dose increased in proportion to the plasma methotrexate concentration (see the recommendations by Bleyer[541]). Hemodialysis and charcoal hemoperfusion have not proved useful for drug removal in patients with renal dysfunction[542,543 and 544] unless they are used repeatedly.[545] The recombinant bacterial enzyme, carboxypeptidase-$G_2$, catabolizes methotrexate to the inactive metabolite, 4-amino-4-deoxy-$N^{10}$-methylpteroic acid (DAMPA), and provides a novel and useful approach to rescuing patients who develop methotrexate nephrotoxicity by providing an alternative route of elimination.[120,546] Carboxypeptidase-$G_2$ is well tolerated and results in a 95.6% to 99.6% reduction in plasma methotrexate concentrations within minutes. Unlike dialysis, there is no rebound of plasma drug levels after carboxypeptidase-$G_2$.

Hepatic toxicity consisting of transient elevations of serum transaminase and, less commonly, hyperbilirubinemia has been associated with standard and high doses of methotrexate but is more common and more severe with high-dose therapy. Hepatic fibrosis has been observed primarily in patients receiving chronic low-dose methotrexate.[520,547] Other side effects include a dermatitis characterized by erythema and desquamation, allergic reactions, and acute pneumonitis.[547,548 and 549] Methotrexate osteopathy is a cumulative toxicity that causes bone pain, osteoporosis, and an increased risk for fractures. Neurotoxicity from high-dose methotrexate includes an acute, stroke-like encephalopathy, seizures, and chronic leukoencephalopathy, particularly in association with cranial irradiation.[281,550,551,552 and 553]

***Resistance***

Mechanisms of resistance to methotrexate identified experimentally include decreased membrane transport, increased levels of the target enzyme DHFR, altered affinity of DHFR for methotrexate, decreased polyglutamation of methotrexate, and decreased thymidylate synthase activity.[494,497,554] Increases in target enzyme levels have been associated with amplification of gene encoding for DHFR, a phenomenon that has also been documented in lymphoblasts from patients whose disease was clinically resistant to methotrexate.[494,555,556] Flow cytometric analysis of lymphoblasts from 29 children with newly diagnosed and relapsed ALL demonstrated heterogeneous expression of elevated DHFR in 11 of 29 specimens and impaired methotrexate transport in 3 of 29 specimens.[557] Newly diagnosed patients whose marrow specimens contained DHFR overproducing subpopulations of lymphoblasts had shorter remission durations than comparable patients whose lymphoblasts only expressed lower DHFR levels. Impaired methotrexate uptake and decreased expression of the reduced folate carrier, which is the membrane transport protein involved in cellular uptake of methotrexate, have been observed in tumor specimens from patients with ALL and osteosarcoma.[558,559 and 560]

***Drug Interactions***

Several drugs have been associated with increased toxicity when coadministered with methotrexate.[87,143,144] The most significant interactions involve agents that interfere with methotrexate excretion, primarily by competing for renal tubular secretion. These drugs include probenecid, salicylates, sulfisoxazole, penicillins, and the nonsteroidal antiinflammatories indomethacin, ketoprofen, and ibuprofen.[87,143,561,562 and 563] Nephrotoxic drugs, such as the aminoglycosides and cisplatin, may also alter the clearance of methotrexate.[143] Pharmacodynamic interactions resulting in synergistic cytotoxic effects have been reported with methotrexate and fluorouracil, methotrexate and cytarabine, and methotrexate and asparaginase.[564]

**Thiopurines**

Mercaptopurine and thioguanine are thiol-substituted derivatives of the naturally occurring purine bases hypoxanthine and guanine ( Fig. 10-12). Mercaptopurine has been used in the treatment of ALL for five decades, primarily for the maintenance of remission. It is also used in the treatment of CML, histiocytosis, and inflammatory bowel disease. In standard maintenance regimens, mercaptopurine is administered orally at a dose of 75 to 100 mg per $m^2$ per day with upward or downward dose

adjustments based on the degree of myelosuppression. Ensuring that patients are receiving their maximum tolerated dose of mercaptopurine appears to be an important factor in the outcome for children with ALL.[516] In a retrospective analysis, when the actual dose of mercaptopurine received increased by 22% as a result of more aggressive prescribing guidelines, the relapse-free survival improved by 18%. [565] High-dose intravenous infusions of mercaptopurine (1,000 mg per $m^2$ over 6 to 24 hours) have also been evaluated as an approach to circumvent the pharmacokinetic limitations of oral dosing. [566,567,568 and 569] Thioguanine is used in the treatment of acute nonlymphocytic leukemia and is administered orally in doses of 75 to 100 mg per $m^2$ daily for 5 to 7 days or in doses of 40 to 60 mg per $m^2$ daily for more prolonged courses. Thioguanine is also currently being studied as a maintenance agent for ALL, [570] and thioguanine infusional therapy has been investigated. [571,572]

The thiopurines are prodrugs that must be converted intracellularly to thioguanine nucleotides to exert a cytotoxic effect. The metabolic pathways for activation of mercaptopurine and thioguanine are outlined in Figure 10-14. The active intracellular metabolites are phosphorylated thiopurine nucleotides, which inhibit de novo purine synthesis and purine interconversion and are incorporated into DNA. [573,574 and 575] Incorporation of thioguanosine into DNA appears to be the critical determinant of thiopurine cytotoxicity.[576] Thioguanine is tenfold more potent and less schedule dependent than mercaptopurine against lymphoblastic leukemia cell lines and lymphoblasts from patients with ALL *in vitro*.[577]

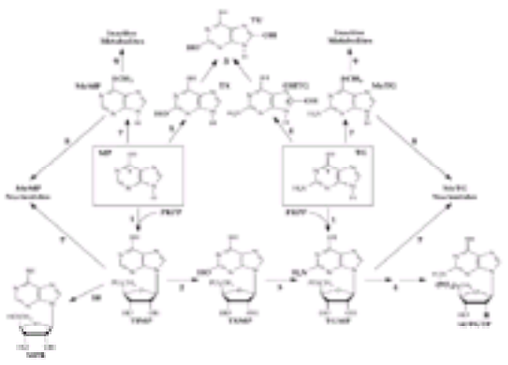


**FIGURE 10-14.** Metabolic pathways of mercaptopurine (MP) and thioguanine (TG). Intracellular activation of these prodrugs involves conversion to thioguanine nucleotides. For MP, this is a three-step process, starting with conversion to thioinosine monophosphate (TIMP), catalyzed by the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) (1). Phosphoribosylpyrophosphate (PRPP) is a required cofactor in this reaction. TIMP is converted to thioxanthosine monophosphate (TXMP) by inosine monophosphate dehydrogenase (2) and then to thioguanine monophosphate (TGMP) by guanosine monophosphate synthetase (3). Thioguanine is converted directly to TGMP by HGPRT. TGMP is phosphorylated by kinases to TGTP (R = OH) and converted to the deoxyribonucleotide dTGTP (R = H) by ribonucleotide reductase (4). dTGTP can then be incorporated into DNA (not shown). There are competing catabolic pathways for the thiopurines, including oxidation to the inactive metabolite, thiouric acid (TU). For MP, this is catalyzed in a two-step reaction by xanthine oxidase (5). The initial oxidation step from TG to 8-hydroxy-thioguanine (OHTG) is catalyzed by aldehyde oxidase (6). The thiopurines also undergo S-methylation, catalyzed by thiopurine methyltransferase (TPMT) (7). Methylmercaptopurine (MeMP) and methylthioguanine (MeTG) can be converted to methylated thionucleotides along the same pathways as the parent drug (8). TPMT can also convert TIMP and TGMP to methylated thionucleotides. MeMP and MeTG can also be oxidized or desulfurated to inactivate metabolites (9). Dephosphorylation of TIMP to mercaptopurine riboside (MPR) is another inactivating step that is catalyzed by several intracellular enzymes (10).

The common toxic effects of mercaptopurine include myelosuppression, hepatic dysfunction (e.g., elevated transaminases and cholestatic jaundice), and mucositis. Myelosuppression is also the primary toxic effect of thioguanine, but chronic administration of oral thioguanine is associated with the development of hepatic VOD. [578]

***Biotransformation***

The thiopurines are extensively metabolized *in vivo* to active and inactive metabolites (Fig. 10-14).[576,579,580,581 and 582] The activation pathway for thioguanine, which is converted to the nucleotide thioguanosine monophosphate in a single step, is more direct than that for mercaptopurine, which undergoes a three-step conversion to the thioguanine nucleotide. The primary degradative pathway for mercaptopurine is conversion to the inactive metabolite thiouric acid by the enzyme xanthine oxidase. The oxidation of thioguanine to thiouric acid follows a different metabolic pathway. Thioguanine is initially converted by aldehyde oxidase to 8-hydroxy-thioguanine, which is the primary circulating metabolite of thioguanine. [573,582]

The thiopurines are also subject to S-methylation by the enzyme thiopurine methyltransferase (TPMT). [583] The level of intracellular TPMT activity is an important determinant of the availability of thiopurines for conversion to active thioguanine nucleotides, and, as a result, TPMT regulates the cytotoxic effect of these thiopurines.[97] The activity of this enzyme is controlled by a common genetic polymorphism, resulting in a trimodal distribution of intracellular enzyme levels ( Fig. 10-15).[97,576] One in 300 patients is deficient of TPMT activity and extremely sensitive to the cytotoxic effects of mercaptopurine, thioguanine, and azathioprine. Even a short course of therapy can result in profound myelosuppression, and erythrocyte thioguanine nucleotide levels in these TPMT-deficient patients are markedly elevated (Fig. 10-15).[584,585 and 586] Very low doses of mercaptopurine (5% to 10% of the standard dose) are tolerable in TPMT-deficient patients, but these doses yield erythrocyte thioguanine nucleotide levels higher than those typically found in patients with normal TPMT levels receiving standard doses of mercaptopurine. [585,586,587 and 588] The 10% of patients who are heterozygous at the TPMT locus and have intermediate enzyme activity levels require more mercaptopurine dose reductions for toxicity than homozygous patients with full enzyme activity.[589] TMPT activity is also inversely related to the erythrocyte thioguanine nucleotide level and the severity of neutropenia, suggesting that TPMT modulates the cytotoxic effect of mercaptopurine.[590] Consideration should be given to measuring erythrocyte TPMT levels in patients who are exquisitely sensitive to the toxic effects of thiopurines.

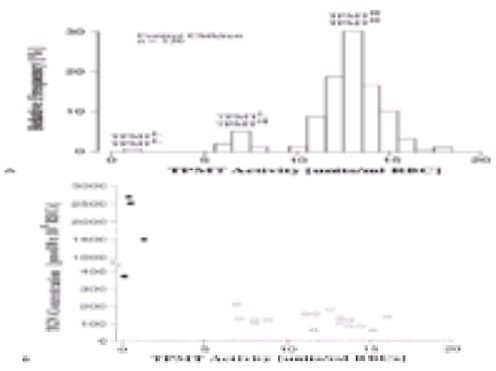


**FIGURE 10-15. A:** Frequency distribution histogram of erythrocyte thiopurine methyltransferase (TPMT) activity in 130 unrelated control children, demonstrating a trimodal distribution resulting from a genetic polymorphism (i.e., a low activity allele, $TPMT^L$, and a high activity allele, $TPMT^H$, at a single genetic locus). One in 300 subjects are TPMT-deficient ($TPMT^L/TPMT^L$ genotype), 11% have intermediate activity ($TPMT^H/TPMT^L$ genotype), and 89% have high activity ($TPMT^H/TPMT^H$ genotype). Within the predominant high-activity population, there is a wide range of TPMT activity. (From Lennard L, Lilleyman JS, Van Loon J, Weinshilboum RM. Genetic variation in response to 6-mercaptopurine for childhood acute lymphoblastic leukaemia. Lancet 1990;336:225.) **B:** Erythrocyte (RBC) thioguanine nucleotide (TGN) levels relative to erythrocyte TPMT activity in four TPMT-deficient patients ( *closed circles*) and 16 control patients (*open circles*) receiving azathioprine. Azathioprine is spontaneously converted to mercaptopurine *in vivo*. The four patients with very low or undetectable TPMT activity experienced severe myelosuppression and had elevated RBC TGN levels compared with controls treated with the same dose. (From Lennard L, Van Loon JA, Weinshilboum RM. Pharmacogenetics of acute azathioprine toxicity: relationship to thiopurine methyltransferase genetic polymorphism. Clin Pharmacol Ther 1989;46:149.)

### *Pharmacokinetics*

The bioavailability of oral mercaptopurine is less than 20%, and the resulting plasma drug concentrations are highly variable ( Fig. 10-13).[81,591] Only one-third of patients achieve plasma concentrations of mercaptopurine above the minimal *in vitro* cytotoxic concentration of 1 µM.[591] Substantial interpatient variability after oral dosing has been confirmed by other investigators.[81,592,593] The median peak plasma concentration was 0.59 µM (range, 0.13 to 2.30 µM) and the median AUC was 1.8 µM • hour (range, 0.39 to 4.80 µM • hour) at oral doses ranging from 65 to 85 mg per $m^2$.[81] Plasma mercaptopurine AUC is not predictive of erythrocyte thioguanine nucleotide levels.[81] Significant intrapatient variability has also been observed in patients monitored after multiple doses over the course of maintenance therapy.[81] The bioavailability of mercaptopurine is limited by the extensive first-pass metabolism of the drug by xanthine oxidase in the liver and intestinal mucosa. When mercaptopurine is coadministered with the xanthine oxidase inhibitor allopurinol, the fraction of the dose absorbed increases fivefold.[594]

The bioavailability of oral thioguanine is also poor, and plasma concentrations are variable, with a 30- to 50-fold range in peak plasma concentration and AUC.[595,596] and[597] The mean (± SD) peak plasma concentration was 0.46 ± 0.68 µM, and the AUC ranged from 0.18 µM • hour to 9.5 µM • hour.[597] These plasma concentrations were within the range required *in vitro* to produce cytotoxicity.[577] The bioavailability was diminished further in non-fasting patients and patients experiencing nausea and vomiting.[596] Plasma thioguanine AUC after an oral does did not correlate with erythrocyte thioguanine nucleotide levels. to produce cytotoxicity.[597]

Mercaptopurine is eliminated primarily by biotransformation. However, renal excretion of unchanged drug does become quantitatively significant (20% to 40%) with high intravenous doses.[567,598] Elimination is rapid, with a total clearance rate of 800 mL per minute per $m^2$ and a half-life of less than 1 hour.[591,594] The intravenous infusion of 50 mg per $m^2$ per hour achieves steady-state plasma drug concentrations in excess of 5 µM and CSF concentrations greater than 1 µM, above concentrations of the drug known to be cytotoxic *in vitro*.

Intravenous administration of thioguanine has been evaluated on single-dose, daily-for-5-days, and continuous infusion schedules.[571,597,599,600] With standard doses (less than or equal to 100 mg per $m^2$), the drug is cleared rapidly. Total clearance is greater than 2,000 mL per minute per $m^2$, and the terminal half-life is 20 minutes.[599] However, a reduced clearance rate (320 mL per minute per $m^2$) and a longer terminal half-life (2 to 6 hours) at doses exceeding 700 mg per $m^2$ indicate that the pharmacokinetics of this drug are dose dependent.[599,600] Similarly, on the continuous infusion regimen, thioguanine clearance declined from 1,370 to 575 mL per minute per $m^2$ and the half-life increased from 2.2 to 5.1 hours when infusion rate was escalated from 10 to 20 mg per $m^2$ per hour.[571] Thioguanine is cleared primarily by biotransformation, with only small amounts of the drug excreted unchanged in the urine.[601] The primary circulating metabolite is 8-hydroxy-thioguanine.[582] Saturation of the degradative enzymes at high doses probably accounts for the dose-dependent pharmacokinetics. Thioguanine penetration into the CSF during the continuous intravenous infusion was 18%.[597]

Erythrocyte levels of thiopurine-derived thioguanine nucleotides have been monitored in children receiving oral therapy. As with plasma concentrations of the parent drug, the erythrocyte metabolite levels are highly variable.[81] Erythrocyte thioguanine nucleotide levels have been correlated with the degree of myelosuppression and the risk of relapse.[602,603] and[604] Children with ALL whose erythrocyte thioguanine nucleotide levels were below the median value of 284 pmol per 8 • $10^8$ erythrocytes had a twofold higher relapse rate than patients with values above the median.[603,604] However, the differences in erythrocyte thioguanine nucleotide levels in the remission and relapse patients were small, and the range of values overlapped completely (100 to 1,300 pmol per 8 • $10^8$ erythrocytes in remission patients and 100 to 800 pmol per 8 • $10^8$ erythrocytes in relapsed patients).[603,604] In other studies, no relationship was found between thioguanine nucleotide levels and outcome.[81,516] Erythrocyte thioguanine nucleotide levels in patients receiving daily oral thioguanine are fivefold higher than nucleotide metabolite levels from mercaptopurine therapy.[570,597,605,606]

### *Drug Interactions*

The classic example of a drug interaction in cancer chemotherapy is the effect of the xanthine oxidase inhibitor allopurinol on the catabolism of mercaptopurine to thiouric acid. When these two agents are administered concurrently, the hematologic toxicity of mercaptopurine is significantly enhanced.[607] Allopurinol pretreatment results in a fivefold increase in bioavailability of the oral dose of mercaptopurine.[594] However, the disposition of intravenously administered mercaptopurine is not affected by the coadministration of allopurinol.[567,594,598] This differential effect results from inhibition of first-pass metabolism of orally-administered mercaptopurine by allopurinol.[594] The dose of oral mercaptopurine should be reduced by 75% when administered to patients receiving allopurinol, but intravenous mercaptopurine doses need not be modified unless the patient has renal dysfunction. The renal excretion of unchanged mercaptopurine increases from 20% to more than 40% of the dose in patients who receive the drug by intravenous infusion in combination with allopurinol.[567] Although allopurinol enhances the bioavailability of mercaptopurine, it has not been shown to augment the antileukemic effect of the drug and may antagonize the antitumor effects on a pharmacodynamic level.[143,594] Because the first step in the oxidation of thioguanine is catalyzed by aldehyde oxidase rather than xanthine oxidase, the coadministration of allopurinol and thioguanine does not require a dose modification. Methotrexate and folates also inhibit xanthine oxidase, and methotrexate can minimally enhance mercaptopurine bioavailability.[608,609]

## Pyrimidine Analogs

### *Cytarabine*

Cytarabine (cytosine arabinoside, ara-C) is an arabinose nucleoside analog of deoxycytidine ( Fig. 10-12) that is active in the treatment of the acute leukemias and lymphoma. After intracellular metabolic activation, cytarabine interferes with DNA replication and repair through inhibition of DNA polymerase a and through incorporation into DNA.[610,611,612] and[613] Depending on the dose and schedule of cytarabine used, incorporation into DNA is thought to inhibit chain elongation, result in chain termination or reinitiation at sites of previously replicated segments, or cause DNA strand breaks.[610,611] and[612] Inhibition of DNA synthesis or incorporation into DNA by cytosine arabinoside triphosphate (ara-CTP) can only occur during the DNA synthesis phase (S phase) of the cell cycle, and more prolonged exposure to cytarabine allows the drug to be incorporated into a larger fraction of the cells as they pass through S phase. Cytarabine incorporation into DNA may also be enhanced by timed retreatment with cytarabine after recruitment of leukemic cells into an active phase of DNA synthesis after the first treatment cycle and the simultaneous administration of cytarabine and colony-stimulating factors, which stimulate leukemic cells into S phase.[614,615,616] and[617]

A wide range of doses and schedules for cytarabine have been used. The standard dose is 100 to 200 mg per $m^2$ as a bolus injection every 12 hours or by continuous infusion, and it is usually administered daily for 5 to 7 days. High-dose regimens (3 g per $m^2$ every 12 hours for four to 12 doses or as a continuous infusion) have also been used with the intention of overcoming resistance mechanisms.[618,619] Low-dose cytarabine regimens (5 to 20 mg per $m^2$ per day over several weeks) are used for the treatment of myelodysplastic syndromes.[620]

#### Biotransformation

After entering cells by the carrier-mediated nucleoside transport system, cytarabine is converted to the active nucleotide, ara-CTP, by three sequential phosphorylations catalyzed by intracellular kinases.[610,621] Ara-CTP then competes with the natural substrate deoxycytidine triphosphate for DNA replicative and repair enzymes. Cytarabine and ara-cytidine monophosphate can also be catabolized to the inactive by-products uridine arabinoside (ara-U) and ara-uridine monophosphate (ara-UMP) by deaminases that are present in high concentrations within cells.[622,623] Cytidine deaminase is a ubiquitous enzyme, and the catabolism of cytarabine to ara-U is the primary route of elimination for the drug. Alterations in these uptake and metabolic pathways within the cancer cell can result in drug resistance, such as a decrease in membrane transport, a decrease in activation by deoxycytidine kinase, an increase in degradation by cytidine deaminase, and an increase in the competing natural substrate, deoxycytidine triphosphate.[610,611,618]

#### Pharmacokinetics

The pharmacokinetic behavior of cytarabine is directly related to the activity of the major degradative enzyme, cytidine deaminase. The bioavailability of oral cytarabine is less than 20% because of extensive presystemic metabolism by high levels of this enzyme in gastrointestinal epithelium and liver.[624] The hepatic extraction ratio for cytarabine is estimated to be as high as 80%.[625] Subcutaneously injected cytarabine is completely absorbed.[624]

Drug elimination is rapid with intravenous dosing. Total clearance is 1,000 mL per minute per $m^2$ or greater, and the postdistributive half-life is 2 to 3 hours.[619,623,624,626,627] Metabolism to ara-U accounts for 80% to 90% of total cytarabine clearance, and renal clearance accounts for less than 10% of total clearance. The ara-U formed is excreted in the urine. Because of the ubiquity of cytidine deaminase (e.g., liver, gastrointestinal tract, plasma, leukocytes), hepatic dysfunction does not significantly alter the rate of elimination of cytarabine. With high-dose prolonged intravenous infusions, the mean steady-state plasma concentration of cytarabine was 5 µM at a dose of 2 g per $m^2$ per day, and the steady-state concentration of ara-U was tenfold higher (60 µM). [619] In these patients, plasma clearance appeared to decrease with increasing dose, suggesting saturation of deaminases at the higher dose levels. [619,628] In children receiving an infusion of 5 g per $m^2$ per day, total clearance was 555 mL per minute per $m^2$,[628] and at steady state, ara-U plasma concentrations are more than tenfold higher than steady-state cytarabine concentrations in children.[629]

The pharmacokinetics of cellular ara-CTP, the active intracellular metabolite of cytarabine, have been characterized in the leukemic blasts from patients receiving high-dose cytarabine. After a 3 g per $m^2$ dose administered as a short infusion, there was considerable interpatient variability in the amount of ara-CTP accumulated in these blasts. There was no correlation between the pharmacokinetics of the parent drug in plasma and the cellular concentrations of ara-CTP in leukemic blasts.[630,631] Patients responding to the drug had a significantly slower rate of elimination of ara-CTP. The half-life of ara-CTP was 5.6 hours in responding patients and 3.2 hours in resistant patients. Responding patients also had significantly higher trough concentrations (196 µM versus 23 µM). [630] This is consistent with earlier studies demonstrating greater *in vitro* ara-CTP formation in the leukemic blasts from patients achieving a complete remission on cytarabine therapy compared with nonresponders.[632] The pharmacokinetics of the active intracellular metabolite appear to be more predictive of outcome than plasma concentrations of the parent drug. The cellular retention of ara-CTP in leukemic blasts was shorter in blasts from patients with T-cell ALL and acute nonlymphoblastic leukemia compared to non–T-cell ALL.[633]

**Toxicity**

The primary toxicities of cytarabine are myelosuppression, nausea and vomiting, and gastrointestinal mucosal damage, including life-threatening bowel necrosis.[610,634,635 and 636] A syndrome of high fever, malaise, myalgias, joint or bone pain, rash, conjunctivitis, and chest pain has also been reported in children receiving cytarabine in standard doses.[637] Coadministration of corticosteroids appears to relieve these symptoms. Neurotoxicity from cytarabine has been primarily associated with high-dose therapy.[281,638,639,640 and 641] The most common manifestation of neurotoxicity is an acute cerebellar syndrome manifesting 3 to 8 days after initiation of therapy, but seizures and encephalopathy have also been reported. [281,640] Nystagmus, ataxia, dysarthria, dysmetria, and dysdiadochokinesia are the classic cerebellar manifestations. In most cases, these neurologic symptoms resolve within a week, but as many as 30% of patients do not regain full cerebellar function.[281,640] Neuropathologic findings include loss of Purkinje's cells and a reactive gliosis in the cerebellum. In addition to dose, other risk factors for the development of neurotoxicity include advanced age and hepatic or renal dysfunction. [640,642,643 and 644] Lowering the dose of cytarabine from 3,000 to 2,000 mg per $m^2$ and administering the drug daily instead of every 12 hours is recommended for patients with renal dysfunction. [644] The drug should be immediately withdrawn if nystagmus or ataxia occurs. Skin and ocular toxic effects have also been observed on high-dose regimens. [636]

***Fluorouracil***

The fluorinated pyrimidine fluorouracil ( Fig. 10-12) is one of the few rationally designed anticancer drugs. It has been widely used in the treatment of carcinomas of the gastrointestinal tract, breast, ovary, and head and neck but it is not used for the treatment of the common childhood cancers. Fluorouracil is administered intravenously as a bolus injection (500 mg per $m^2$), usually on a daily-for-5-days schedule, or as a continuous infusion (800 to 1,200 mg per $m^2$ over 24 hours). Protracted low-dose infusional fluorouracil is also effective and well tolerated. [645,646]

Fluorouracil is a prodrug and must be converted intracellularly to nucleotides before expressing cytotoxicity. [647,648 and 649] There are several possible pathways for the anabolism of fluorouracil to active intracellular metabolites, and the relative importance of each pathway is tissue- and tumor-dependent. [650] The deoxyribonucleotide 5-fluorodeoxyuridine monophosphate (5-FdUMP) is a potent inhibitor of thymidylate synthase, leading to depletion of the DNA precursor, thymidine; and the ribonucleotide fluorouridine triphosphate is incorporated into RNA. Inhibition of thymidylate synthase by FdUMP is thought to be the primary mechanism of action in most tumors.[651] Mechanisms of resistance to fluorouracil in preclinical models include an increase in intracellular catabolism by DPD, a decrease in the activity of activating enzymes, and an increase in levels of the target enzyme thymidylate synthase. [652,653 and 654] A mutant thymidylate synthase with decreased affinity for FdUMP has also been described.[655]

**Pharmacokinetics**

Bioavailability of oral fluorouracil is highly variable, in part because of a saturable first-pass elimination process. [656,657] In one study of 13 patients, two had undetectable plasma drug levels after an oral dose of 750 mg, and the fraction of the dose absorbed ranged from 0% to 74%. [657] Because of this erratic absorption, fluorouracil should not be administered by the oral route, unless it is administered with agents, such as eniluracil, [658] which blocks the presystemic catabolism of fluorouracil and enhances its bioavailability. Bioavailable fluorouracil prodrugs, such as capecitabine and tegafur-uracil, have been also developed for oral administration. These agents are converted to fluorouracil after absorption and provide more prolonged drug exposure, similar to a prolonged intravenous infusion.[659,660,661 and 662] Subcutaneously administered fluorouracil is well tolerated and has nearly complete bioavailability. [663]

Fluorouracil is eliminated primarily by biotransformation. The degradative pathway is the same as that for the naturally occurring pyrimidines uracil and thymine. [648,658] Less than 10% of the drug is excreted unchanged in the urine. With standard bolus dosing, the elimination of fluorouracil is rapid. The half-life is 6 to 20 minutes, and total clearance is greater than 1,000 mL per minute.[649,658,664,665] With a continuous infusion schedule, the pharmacokinetics differ significantly, with clearance values as high as 5,000 mL per minute. In children treated with 80 mg per $m^2$ per hour for 12 hours, the mean steady-state concentration of fluorouracil was 6.7 µM, and the clearance was 2,500 mL per minute per $m^2$.[666] This schedule-dependent clearance is consistent with a dose-dependent or saturable clearance process. [649,664,667,668] Although the liver is thought to be the principal site of drug catabolism, the high clearance values with infusions exceed the rate of hepatic blood flow, indicating that biotransformation must also be taking place in other organs.[664]

Circadian dependency of fluorouracil toxicity appears to be related to rhythmic threefold to 25-fold fluctuations in plasma drug concentrations over the course of the day.[647,669,670,671 and 672] During a continuous intravenous infusion, the plasma fluorouracil concentrations were highest in the late morning and lowest shortly before midnight; and plasma concentrations of fluorouracil were inversely related to the activity of the catabolic enzyme, DPD, in peripheral blood mononuclear cells. [670,671] Adjustments in the dose or rate of fluorouracil infusion may be indicated based on the time of day the drug is being administered. [672,673]

**Toxicity**

The incidence and severity of clinical toxicities of fluorouracil depend on the dosing schedule. With intravenous bolus dosing, myelosuppression is the primary toxicity, but if the drug is given as a continuous infusion at doses up to 14,000 mg over 24 hours, myelosuppression is less prominent and stomatitis and diarrhea become dose-limiting.[674] Protracted low-dose infusions can produce palmar-plantar dysesthesia (hand-foot syndrome). Reversible neurologic toxicity characterized by somnolence, cerebellar ataxia, and headache; ocular toxicity consisting of conjunctivitis and ectropion; dermatitis; and, rarely, cardiotoxicity, which can include chest pain, arrhythmias, and ischemic changes on electrocardiogram, are also reported. [647] Inherited partial deficiency of the catabolic enzyme DPD in 1% to 3% of the population is associated with severe fluorouracil toxicity. [101,654,675,676] Fluorouracil half-life is markedly prolonged, and there is no evidence of catabolism in DPD-deficient patients.[675]

**Drug Interactions**

Folates are required for the stable binding of 5-FdUMP to its target enzyme, thymidylate synthase, and the combination of leucovorin and fluorouracil is synergistic in experimental systems.[677] This combination has been studied in a large number of clinical trials, primarily in adults with gastrointestinal tumors. In randomized clinical trials, the combination results in higher response rates than fluorouracil alone. [677] Pediatric trials of fluorouracil and leucovorin have failed to demonstrate substantial

activity against sarcomas.[666,678] Other modulators of the fluorouracil anticancer effect include the interferons, cisplatin, zidovudine, and antifolates. [646,677,679,680 and 681]

## ANTITUMOR ANTIBIOTICS

Most of the current antitumor antibiotics (Fig. 10-16) are natural products that were originally isolated from the microbial broth of a variety of species of the group of soil microorganisms, *Streptomyces*. The agents from this class of anticancer drugs that are used in the treatment of childhood cancers include the anthracyclines, mitoxantrone, dactinomycin, and bleomycin.

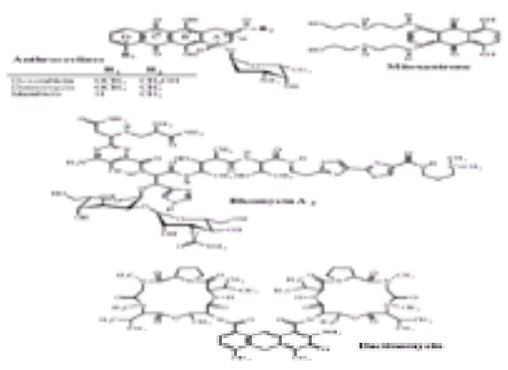


**FIGURE 10-16.** Chemical structures of the antitumor antibiotics commonly used in the treatment of childhood cancers: the anthracyclines, doxorubicin, daunomycin, and idarubicin; mitoxantrone; bleomycin $A_2$; and dactinomycin. Doxorubicin and daunomycin differ only in the presence or absence of a hydroxyl group on the carbonyl side chain at ring position 9. The structure of idarubicin (4-demethoxydaunomycin) is identical to daunomycin except for the absence of a methoxy group at ring position 4. Mitoxantrone differs from the anthracyclines by virtue of its three-ring nucleus, its symmetric aminoalkyl side chains, and its lack of a glycosidic substituent, which is important for water solubility of the anthracyclines.

The antitumor antibiotics avidly bind to DNA by a process called *intercalation*, in which a planar, multiring portion of the drug inserts between base pairs of the DNA double helix. The anthracyclines mitoxantrone and dactinomycin interfere with the topoisomerases, nuclear enzymes that regulate the three-dimensional shape of DNA by cleaving and religating DNA during replication, transcription, repair, and recombination. Intercalators like the anthracyclines produce protein-associated DNA strand breaks, apparently by interfering with this cleavage-religating process. [55,56 and 57] Alterations in the activity or function of the topoisomerases may be one mechanism of multidrug resistance.[682,683]

### Anthracyclines

The anthracyclines doxorubicin, daunomycin (daunorubicin), and idarubicin are highly pigmented compounds composed of a planar tetracyclic anthraquinone nucleus linked to the amino sugar daunosamine (Fig. 10-16). Doxorubicin has a wide range of clinical activity against pediatric cancers, including the acute leukemias, lymphomas, sarcomas of soft tissue and bone, Wilms' tumor, neuroblastoma, and hepatoblastoma. The use of daunomycin and idarubicin is currently limited to the acute leukemias.

The mechanism of anthracycline antitumor activity may be multifactorial. [682,684,685,686,687,688 and 689] These agents intercalate into DNA and induce topoisomerase II–mediated single- and double-strand breaks in DNA. Topoisomerase II–mediated DNA cleavage may occur by nonintercalative mechanisms. [684] In addition, anthracyclines block helicase-catalyzed dissociation of duplex DNA into single strands and oxidize DNA bases. [684] The anthracyclines can undergo chemical reduction through several enzymatically catalyzed pathways or by interaction with oxymyoglobin in the heart, yielding reactive free radical intermediates ( Fig. 10-17).[684] Transfer of an electron from these unstable radicals to molecular oxygen yields superoxide radicals that can generate hydrogen peroxide and hydroxyl radicals, compounds that can cause oxidative damage to cellular macromolecules. The interaction of anthracyclines with iron plays a role in free radical formation. Anthracyclines may also exert cytotoxic effects through a direct interaction with the cell membrane.[684] Experimental evidence indicates that topoisomerase-mediated DNA damage is the most important mechanism. Resistance to these agents is associated with increased expression of a family of membrane-bound drag transporters which includes P-gp, MRP, breast cancer resistance protein and lung resistance–related protein. [690] Idarubicin appears to be less susceptible to this form of drug resistance than daunomycin *in vitro*, possibly because it is more lipophilic. [691,692] Altered levels or affinity of the target enzyme, topoisomerase II, also confers resistance to the anthracyclines.[684]

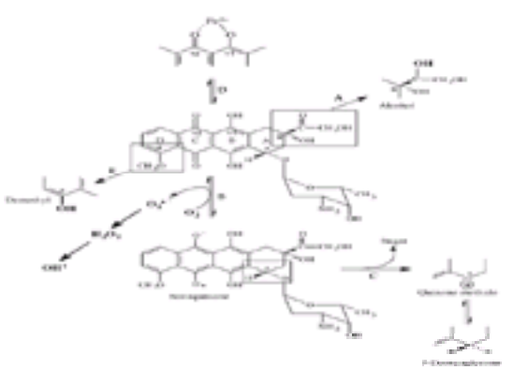


**FIGURE 10-17.** Doxorubicin chemistry. Reduction of the carbonyl group at the 9 position leads to the formation of the alcohol, doxorubicinol (A). The alcohol metabolites of daunomycin and idarubicin are also prominent metabolites of these agents. The alcohol metabolites of doxorubicin and daunomycin retain some cytotoxic activity but are considerably less active than the parent drug, whereas the cytotoxicity of idarubicinol is equivalent to that of the parent drug. Reduction of the C ring, catalyzed by one of several flavoproteins, reductases, or oxymyoglobin, leads to the formation of the semiquinone free radical (B). In the presence of oxygen, the unpaired electron is donated to oxygen to form the highly reactive superoxide radical, and the anthracycline returns to its parent form (i.e., redox-cycling). Superoxide ($O_2^{\bullet -}$) is converted to hydrogen peroxide ($H_2O_2$) by superoxide dismutase, and $H_2O_2$ is reductively cleaved in the presence of iron or doxorubicin semiquinone to hydroxyl radicals ($^{\bullet}OH$), which is destructive to tissues. Under hypoxic conditions, molecular rearrangement leads to the loss of the sugar and formation of an intermediate free radical (not shown) that can react with cellular macromolecules or become further reduced to the inactive 7-deoxyaglycone metabolite, the tautomer of which is quinone methide, an alkylating species (C). This is thought of as a minor pathway that leads to inactivation. Oxygens at ring positions 11 and 12 chelate iron, which is subsequently reduced by cellular reducing systems such as cytochrome P-450 reductase or by auto-oxidation of the hydroquinone (B ring) or, with doxorubicin, oxidation of the ring position 9 side chain (D). Demethylation of the methoxy group at ring position 4 of doxorubicin or daunomycin (E). This hydroxyl group may subsequently be conjugated with glucuronide or sulfate, which detoxifies the drugs.

The anthracyclines are administered by a wide range of schedules, including bolus injection daily, weekly, or every 3 to 4 weeks, short infusions of up to 6 hours; continuous infusion over 24 to 96 hours; and long-term, low-dose infusions over weeks to months. The antitumor effect does not appear to be influenced by these variations in the schedule of administration.[684,693,694] However, schedule can significantly influence toxicity. Administering doxorubicin weekly or by infusion appears to reduce cardiotoxicity and nausea and vomiting but enhances mucositis. [693,695,696 and 697] Doxorubicin has also been administered intraarterially. [698]

#### *Biotransformation*

The principal metabolites of the anthracyclines ( Fig. 10-17) are the corresponding alcohols (13-dihydroderivative), doxorubicinol, daunomycinol, and idarubicinol, formed by the action of aldoketoreductase.[684,699] Doxorubicinol and daunomycinol retain cytotoxic activity but are considerably less active than the parent drugs, but idarubicinol is as potent as idarubicin.[700] Anthracycline free-radical species can be generated by several reactions. One electron reduction to the semiquinone free radical is catalyzed by flavin-based oxidoreductases, including NADPH cytochrome P-450 reductase, NADH dehydrogenase, and xanthine oxidase.[686] If the extra electron in the semiquinone is donated to molecular oxygen, the parent drug is regenerated (redox cycling). Under hypoxic conditions, the semiquinone is converted to a 7-deoxyaglycone metabolite. Although reactive intermediates are formed after elimination of the sugar, this reaction appears to inactivate the drug.[684,685] Deoxyaglycones are minor metabolites of the anthracyclines.[699] Anthracyclines avidly chelate iron; the resulting drug-iron complex can undergo reduction to yield free radicals by cellular reducing systems (e.g., glutathione, NADH cytochrome P-450 reductase) or by auto-oxidation of the anthracycline molecule.[686] Anthracyclines may be inactivated by conjugation with sulfate or glucuronide after demethylation (i.e., doxorubicin and daunomycin) of the methoxy group at the 4 position on the ring ( Fig. 10-17).

***Pharmacokinetics***

Instability of doxorubicin and daunomycin in an acid environment prevents their oral administration. Idarubicin can be administered orally and has a bioavailability of 20% to 30%.[699,701,702 and 703] The severe vesicant properties of the anthracyclines prohibit intramuscular or subcutaneous administration.

After an intravenous injection, there is an initial rapid decline in plasma concentration, which is generally attributed to the rapid and avid binding of these drugs by tissues; the distributive half-life is approximately 10 minutes.[699,704,705,706 and 707] This extensive binding also accounts for the very large volumes of distribution (greater than 500 L per $m^2$). Tissue anthracycline levels can be up to 100-fold higher than plasma drug concentrations, and tissue levels persist longer.[694,708,709] The distributive phase is followed by a long terminal elimination phase, with half-lives of 30 hours for doxorubicin and 15 to 20 hours for daunomycin and idarubicin.[704,707,710,711] Despite the fact that drug concentrations at the start of the terminal phase are 1/50 the peak concentrations, 66% to 80% of the total drug exposure occurs during this terminal phase.[705,706] With prolonged continuous infusion of 4 mg per $m^2$ per day, doxorubicin plasma concentration averaged 6.0 ng per mL, and the plasma concentration was strongly correlated with the degree of leukopenia.[695]

The anthracyclines are eliminated by biotransformation (primarily hepatic) and biliary excretion. Renal excretion accounts for only 5% to 15% of total drug clearance.[707,710,711] The total clearance exceeds 500 mL per minute per $m^2$, and in children, clearance corrected for body surface area is independent of age.[698,705,707,711] The pharmacokinetic parameters appear to be linear over a wide range of dosage schedules.[712,713]

Dosage modifications are usually not required for doxorubicin and daunomycin in patients with renal dysfunction, although doxorubicin clearance is delayed in patients on hemodialysis.[714] Idarubicin clearance is correlated with creatinine clearance and appears to be reduced with renal impairment.[715] Delayed clearance of doxorubicin associated with an increase in myelosuppression and mucositis has been reported in adults and children with hepatic dysfunction.[707,716,717 and 718] However, abnormal liver function test results do not correlate well with doxorubicin clearance.[110,719,720] The best recommendation is that dose reduction be reserved for patients with multiple liver function test abnormalities or direct bilirubin elevations greater than 2.0 mg per dL, although this guideline may significantly underdose some patients.[110,686,721] Modification of anthracycline dose may also be indicated in obese patients (greater than 130% of ideal body weight), because they appear to eliminate doxorubicin slower and therefore have a twofold higher drug exposure (i.e., AUC) than nonobese patients receiving the same dose.[722]

The alcohol metabolites of the anthracyclines are also detectable in plasma. Exposure to doxorubicinol as measured by the AUC is approximately one-half that of doxorubicin.[699,706,723] In contrast, aldoketoreductase has a higher affinity for daunomycin and idarubicin, and the terminal half-lives of daunomycinol (20 to 40 hours) and idarubicinol (50 to 80 hours) are longer than those of their respective parent drugs. As a result, exposure to daunomycinol and idarubicinol is twofold and fivefold higher than to daunomycin and idarubicin, respectively.[698,699,705,711,712,724] There is accumulation of idarubicinol with daily administration (oral or intravenous) of idarubicin.

***Toxicity***

The acute toxicities of the anthracyclines include myelosuppression, mucositis (less prominent with daunomycin), nausea, vomiting, diarrhea, and alopecia.[725] Extravasation of these agents leads to severe local tissue damage and deep ulcerations, which heal very slowly and are difficult to skin graft. Extravasation has traditionally been managed with ice packs and local injection of corticosteroids and sodium bicarbonate, but these measures have only marginal benefit. In a prospective clinical trial of topical DMSO (99% solution), no ulcerations occurred in 20 patients with suspected anthracycline extravasation when DMSO was applied twice and allowed to air dry, six times per day for 14 days.[726] In severe cases, consideration should be given to early surgical excision of the affected tissues followed by full-thickness skin graft or skin flap coverage.[727]

Anthracyclines can also potentiate radiation reactions in many tissues, including skin, liver, esophagus, lungs, and heart, and the concurrent use of these two modalities should be avoided. A radiation recall phenomenon can be observed if an anthracycline is administered in the postirradiation period.[725]

Anthracyclines can cause acute and chronic cardiac toxicity.[728,729] The acute form is characterized by arrhythmias and conduction abnormalities, but there can also be an acute drop in left ventricular function, reaching a nadir at 24 hours, followed by variable recovery.[730] Rarely, this acute toxicity is manifested as the myocarditis-pericarditis syndrome, which in its severest form is characterized by the rapid onset of congestive failure associated with pericarditis.[731] In general, the acute asymptomatic cardiac changes are transient and do not prevent further use of anthracyclines.[684]

Chronic cardiomyopathy can be separated into an early form, which occurs during treatment or within 1 year of completing anthracyclines, and a late form, which occurs more than a year after completing anthracyclines.[728,732] The early form of cardiomyopathy is related to the cumulative dose of anthracyclines. The incidence of clinically apparent congestive heart failure starts increasing after cumulative doses exceed 450 mg per $m^2$ for doxorubicin and 700 mg per $m^2$ for daunomycin.[733,734] A maximum lifetime safe cumulative dose has not been defined for idarubicin, but cumulative doses of up to 150 mg per $m^2$ appear to be well tolerated.[735] Other factors that are reported to increase the risk for the development of a cardiomyopathy include a high dose rate (bolus or short infusion) of doxorubicin administration, prior or concurrent mediastinal irradiation, and preexisting cardiac disease. Children appear to be at higher risk for cardiac toxicity, and those younger than 5 years are at higher risk than older children.[725,736,737] Girls have a significantly higher incidence of abnormal cardiac findings at any given cumulative dose of doxorubicin than boys.[735,737,738] Lowering peak concentrations of anthracycline by administering the drugs on a lower-dose weekly schedule or by 6- to 96-hour continuous infusions appears to reduce the cardiotoxic effects without compromising the antitumor effect.[694,697,739,740 and 741] A daily divided dosing schedule of doxorubicin was as cardiotoxic as a single bolus dose.[742]

Late cardiotoxicity appears to be more common in children[743,744,745 and 746] than in adults and is characterized by a progressive decrease in fractional shortening, left ventricular mass and wall thickness relative to body size, and an increase in left ventricular afterload.[732,743] The probability of abnormal cardiac function is associated with young age at diagnosis, female sex, dose rates of greater than or equal to 50 mg per $m^2$, and cumulative doses greater than or equal to 550 mg per $m^2$. The heart appears unable to grow in proportion to the child, leading to a small, poorly compliant left ventricle. Prolonged infusions of anthracyclines may not protect children from late cardiotoxicity.[732] Although late anthracycline cardiotoxicity was initially thought to occur primarily in children who received a cumulative doxorubicin dose greater than or equal to 300 mg per $m^2$,[747] sporadic cases with lower cumulative doses were reported,[737,744,745 and 746] and a recent long-term follow-up study in children who were 7 to 14 years out from treatment confirmed that cardiac abnormalities occur at lower total doses.[748] Sudden late decline in cardiac function has also been attributed to intercurrent viral infections in patients with diminished cardiac reserve resulting from prior anthracycline therapy.[749]

Late-onset congestive heart failure and late-effects studies showing a high incidence of subclinical cardiac damage and dysrhythmias in children who were previously treated with presumably safe cumulative doses of anthracyclines suggest that children are more sensitive to the cardiotoxic effects of these agents. Maximum, lifetime cumulative dose levels defined in adults may not be applicable to children, especially very young children. In one study, the risk of late cardiotoxicity in children was low if the cumulative anthracycline dose was less than 350 mg per $m^2$ and the echocardiogram at 1 year off therapy was normal.[750] These data indicate that long-term monitoring of cardiac function should be performed in children who were previously treated with anthracyclines.

Serial echocardiography and radionuclide cineangiography can detect subclinical decline in left ventricular function at cumulative anthracycline doses below the maximum lifetime limit, and endomyocardial biopsies show a steady increase in damage to myocytes with increasing cumulative dose. [729] The primary pathologic change in the myocardium is the destruction and loss of myofibrils and sarcoplasmic vacuolation. [751] Myocardial damage appears to result from the generation of free radicals of the drug (Fig. 10-17) or secondary oxygen-free radicals. These highly reactive species can damage lipid biomembranes and cellular organelles. [684] Myocardium has limited ability to withstand this oxidative stress, because of its low levels of catalase, which detoxifies peroxides. In an experimental model, the alcohol metabolites were more cardiotoxic than the parent anthracyclines and have been shown to accumulate in the heart. [708,709,752,753] Cardiac doxorubicin and doxorubicinol concentrations in human autopsy hearts were significantly higher than concentrations in skeletal muscle and smooth muscle organs (i.e., bladder and uterus).[753]

Endomyocardial biopsy is the most sensitive and direct method of assessing the degree of anthracycline-induced cardiomyopathy. [751] The results correlate well with cardiac function measured at cardiac catheterization, but the technique is invasive, expensive, and technically demanding. Conventional noninvasive functional studies, however, such as the electrocardiogram, echocardiogram, and radionuclide cineangiography, may not demonstrate abnormalities until a critical degree of myocardial injury has occurred, and these studies do not appear to be predictive for the late cardiac effects of anthracyclines. [732,753] Cardiac troponin T serum levels and myocardial uptake of radiolabeled antimyosin antibody, which are sensitive and specific measures of myocardial cell injury, are elevated in children treated with anthracyclines and may be predictive for functional outcome after treatment. [754,755,756 and 757]

Children receiving anthracycline therapy should have their cardiac function closely monitored. Echocardiograms or radionuclide cineangiography are generally recommended before starting therapy and then periodically before courses of anthracyclines. A decline in the shortening fraction to less than 28% by echocardiogram or a 15-percentage point decline in left ventricular ejection fraction (LVEF) or an LVEF less than 45% by cineangiography are indications to discontinue anthracycline therapy. However, the optimal method of screening and the use of screening results to determine anthracycline dose modifications remain controversial. [732,758,759 and 760]

Current approaches to the prevention of anthracycline cardiac toxicity include altering the schedule of drug administration to lower peak concentrations, coadministration of agents that protect the myocardium from the toxic effects of anthracyclines, and the development of less cardiotoxic anthracycline analogs, such as idarubicin and epirubicin, the new morpholino anthracycline derivatives, and liposomal formulation of doxorubicin. [729,761,762]

The most promising cardioprotective drug is a chelating agent, dexrazoxane. This drug undergoes hydrolysis intracellularly to a compound that is similar in structure to EDTA and tightly binds iron, a cofactor in anthracycline free-radical reactions. [684,686] Preclinical studies demonstrated the ability of dexrazoxane to block the cardiotoxicity of anthracyclines[763] and led to several clinical trials of this drug in patients who were receiving doxorubicin-containing chemotherapy regimens. [763,764 and 765] The dexrazoxane dose is determined from the doxorubicin dose as a ratio of 10 mg of dexrazoxane for each 1 mg of doxorubicin. Clinical and subclinical cardiac toxicity, as measured by incidence of congestive heart failure, decline in LVEF on radionuclide cineangiography, and endomyocardial biopsy, was significantly reduced in patients receiving dexrazoxane.[130,766,767] The cardioprotective effect of dexrazoxane has also been demonstrated in a randomized trial in children. [765] In most trials, dexrazoxane had no effect on the antitumor activity of doxorubicin.

### *Drug Interactions*

Doxorubicin elimination half-life may be prolonged when coadministered with cyclophosphamide or nitrosoureas, and doxorubicin clearance appears to be enhanced by coadministration with etoposide.[699] The cardioprotectant, dexrazoxane, does not modify doxorubicin pharmacokinetics. [768] Chemosensitizers used to modulate multidrug resistance mediated by P-gp can substantially alter the disposition of the anthracyclines. [171] For example, cyclosporine and its analog valspodar increases the systemic drug exposure (i.e., AUC) of doxorubicin by 50% and doxorubicinol by three- to fourfold. Doxorubicin-related toxicity is also significantly enhanced when the drug was administered in combination with cyclosporine.[769,770 and 771] The mechanism of this interaction is likely to be related in part to inhibition of P-gp in the biliary tract and decreased excretion of doxorubicin and doxorubicinol into the bile.

## Mitoxantrone

Mitoxantrone is a synthetic anthracenedione that has a planar tricyclic nucleus with two symmetric paraaminoalkyl side chains, but no glycosidic substituent ( Fig. 10-16).[684] Mitoxantrone induces topoisomerase II–mediated DNA strand breaks similar to the anthracyclines,[772,773] but it has a diminished capacity to undergo redox reactions compared to the anthracyclines. As a result, it does not appear to induce significant free-radical tissue injury, which is believed to be the mechanism of anthracycline cardiomyopathy.[684] Mitoxantrone is currently used in salvage regimens for the acute leukemias and lymphomas.[774,775 and 776] It is usually administered on a daily for 3 to 5 days, weekly, or every 3-week schedule.[777,778]

The plasma concentration-time profile of mitoxantrone resembles that of the anthracyclines, with an initial rapid decline ($t_{1/2}$, 10 minutes) and a prolonged terminal elimination phase ($t_{1/2}$, greater than 24 hours).[773] Mitoxantrone is metabolized by oxidation of the terminal hydroxyl groups on the side chains to the inactive mono- and dicarboxylic acids.[779] Biliary excretion appears to be a major route of elimination for mitoxantrone, but renal excretion of parent drug accounts for less than 10% of the administered dose.[773] Mitoxantrone is avidly tissue bound. It has a volume of distribution of 500 to greater than 3,000 L per $m^2$ and can be detected in tissues for weeks after a dose.[773,780] Mitoxantrone clearance is variable and ranges from 100 to 500 mL per minute per $m^2$.[773]

The acute toxicities of mitoxantrone include myelosuppression, mucositis, mild nausea and vomiting, diarrhea, and alopecia. Patients may also notice a bluish discoloration of the sclera, fingernails, and urine. Tissue damage from extravasation of mitoxantrone is uncommon. Mitoxantrone is less cardiotoxic than anthracyclines at equivalent myelosuppressive doses in animal models and in some clinical trials, but the long-term cardiac effects in children have not been studied.[781,782 and 783]

## Bleomycin

Bleomycin is a unique antibiotic that is a mixture of 11 low-molecular-weight (1,500 d), water-soluble glycopeptides. The major species is bleomycin $A_2$ (Fig. 10-16), which accounts for 65% of the commercial preparation. Bleomycin chelates divalent redox-active transition metal ions, such as iron, cobalt, zinc, nickel, or copper, but it is only active in the ferrous form.[784,785,786 and 787] The bleomycin-iron complex binds tightly to DNA, with partial intercalation between guanosine-cytosine base pairs. After binding to DNA, the bleomycin-iron complex produces single- and double-strand DNA breaks by a $Fe^{2+}$-$O_2$-catalyzed free radical reaction.[788] The bleomycin • Fe coordination complex oxygenates the C4' hydrogen of deoxyribose, and cuts DNA in the minor groove, predominately at the CpT and GpC sequences in actively transcribed chromatin domains.[784,789]

The primary determinants of bleomycin cytotoxicity are cellular uptake, DNA repair activity, concurrent medications that alter DNA conformation (e.g., intercalating agents), and the level of activity of bleomycin hydrolase. The latter is a cysteine proteinase that is found in normal tissues and tumor cells and that hydrolyzes a terminal carboxamide group to form an inactive metabolite.[784,789,790] Lung and skin, the tissues with the greatest susceptibility to bleomycin damage, have the lowest levels of this enzyme. In contrast, liver, spleen, intestine, and bone marrow, sites that are less susceptible to bleomycin toxicity, have high levels of this enzyme. [785,791] Bleomycin-resistant cells lines have an increased capacity to hydrolyze bleomycin and an enhanced capacity to repair DNA damage. [792,793]

### *Dosage and Toxicity*

Bleomycin can be administered intravenously by bolus or infusion, intramuscularly, or subcutaneously at doses of 10 to 20 U per $m^2$. A unit is a measure of the drug's cytotoxic activity in bacteria and is equivalent to approximately 1.2 to 1.7 mg of peptide. [791] The drug is active against Hodgkin's disease, lymphomas, and testicular cancer and other germ cell tumors. Bleomycin also has been administered regionally into the pleural space for malignant pleural effusions and intravesicularly for bladder tumors.[794,795]

Unlike most other anticancer drugs, bleomycin is not myelosuppressive. The dose-limiting toxicity is an interstitial pneumonitis that can lead to pulmonary fibrosis. Below a total cumulative dose of 450 U, sporadic cases of pulmonary toxicity are reported, with an incidence of 3% to 5%. At cumulative doses above 450 U, the

incidence increases with the dose.[796,797] Patients with this toxicity present with a persistent dry cough and exertional dyspnea that can progress to tachypnea, hypoxia, and death.[796,797] The chest x-ray typically shows reticulonodular infilterates at the base. A decline in the single breath diffusing capacity for carbon monoxide is the most sensitive measure of subclinical damage, but it may not delineate those patients who are at highest risk to develop clinically symptomatic toxicity. [796,797] and [798] Pulmonary irradiation and the use of supplemental oxygen may enhance the risk of pulmonary toxicity in patients receiving bleomycin, [785,797,799] but others have found that serum creatinine and age older than 30 years may be more important predictors of pulmonary toxicity than the dose or exposure to supplemental oxygen. [800,801] and [802] Concurrent use of G-CSF does not appear to enhance bleomycin pulmonary toxicity.[803] The pathologic changes in the lung include edema and cellular infilteration in the perivascular interstitial space, followed by damage to alveolar lining cells and formation of hyaline membranes and fibrosis. [804] These changes may progress even after the drug is stopped. Pulmonary function should be closely monitored in patients receiving bleomycin, and the drug should be discontinued at the first sign of lung damage. High-dose corticosteroids may be of value in decreasing fibroblast activity, although this recommendation is based only on anecdotal experience. [797]

Dermatologic toxicity from bleomycin is common. Linear hyperpigmentation of the skin is the most common finding, but other mucocutaneous reactions include erythema, induration, desquamation, and sclerosis of the skin; alopecia; nail hyperpigmentation and deformities; and mucositis. [805] Other side effects include nausea and vomiting, fever, hypersensitivity reactions, and Raynaud's phenomenon.

***Pharmacokinetics***

Bleomycin is not administered orally as it would probably be enzymatically degraded in the intestinal tract. Absorption after intramuscular and subcutaneous injection is almost complete, and plasma concentrations with a continuous subcutaneous infusion closely simulate those after an intravenous infusion. [785,791,806,807] With intravenous bolus dosing in children, the drug has a biphasic plasma disappearance curve with a terminal half-life of approximately 3 hours. Total clearance was 41 mL per minute per $m^2$, and renal clearance accounted for 65% of total drug clearance. [808] Patients with renal failure have prolonged terminal drug half-lives, higher plasma concentrations, and delayed clearance.[785,791,809,810] Bleomycin clearance is diminished in children previously treated with cisplatin, including those in whom the serum creatinine and blood urea nitrogen levels were not increased. [785,808] Concurrent use of other nephrotoxic drugs may also impair bleomycin elimination and augment its toxicity. A 45% to 65% dosage reduction has been recommended for patients with a creatinine clearance of less than 30 mL per minute per $m^2$.[785] In patients undergoing hemodialysis, bleomycin was not detected in the dialysate. [811]

**Dactinomycin**

Dactinomycin (actinomycin D) was one of the first drugs demonstrated to have significant antitumor activity in humans, and it has been in clinical use for almost 40 years. It continues to have a role in the treatment of Wilms' tumor and rhabdomyosarcoma, but it has been supplanted by the anthracyclines in many treatment regimens. Dactinomycin is composed of a planar tricyclic ring chromophore (phenoxazone) to which two identical cyclic polypeptides are attached ( Fig. 10-16).[812] The drug intercalates between DNA bases, preferentially binding to the base sequence d(ATGCAT). [813] Dactinomycin binding to DNA causes topoisomerase-mediated single- and double-strand breaks in DNA.[682,814] It also blocks the replication and transcription of the DNA template. [812]

There is limited information on the clinical pharmacology of dactinomycin. The drug is administered intravenously, traditionally on a daily-for-5-days schedule at a dose of 15 µg per kg per day. A single bolus dose of 45 to 60 µg per kg is also used for Wilms' tumor, because it is more convenient, it is no more toxic than the daily-for-5-days regimen, and it is equally effective. [44] A daily for 3 days schedule on weeks 1, 2, 4, and 5 was more hepatotoxic.[815]

After an intravenous bolus injection, dactinomycin has an initial rapid disappearance as a result of its avid tissue binding. [816,817] This distributive phase is followed by a prolonged elimination phase with a half-life of approximately 36 hours. [816] The drug is eliminated by renal and biliary excretion, although only 30% of the administered dose is recovered in the urine and stool over the week after a dose. Only a small fraction of the dose appears to be metabolized. [816]

The primary toxicities of dactinomycin are myelosuppression, orointestinal mucositis, and severe nausea and vomiting. Extravasation of this drug can result in severe local tissue damage and ulceration. Hepatic VOD is a potentially fatal toxicity of dactinomycin in patients with Wilms' tumor. VOD usually occurs during the first 10 weeks of treatment and is characterized by fever, hepatomegaly, ascites, weight gain, jaundice, elevated serum transaminases, and thrombocytopenia. [818] The risk of VOD is similar, with 60 µg per kg for 1 day and 15 µg per kg per day for 5 days schedules. [44] The incidence of VOD in Wilms' tumor is approximately 5%, and risk factors include low body mass, young age, and concomitant radiation.[815,818,819] Dactinomycin is a radiation sensitizer that can enhance the local toxicity of radiation therapy if administered concurrently. Potentiation of radiation pneumonitis is especially problematic. [820] It can also cause a radiation recall effect if administered up to 2 years after irradiation.[821]

## PLANT PRODUCTS

Plant products have been used to treat a variety of diseases for hundreds of years and are still an important source of medically useful and illicit drugs. [822] It has been estimated that in recorded history more than 3,000 species of plants have been used as some form of cancer treatment. Despite extensive screening in the modern era of cancer treatment, however, only a few clinically active anticancer drugs have been derived from the higher plants. [823,824] The only plant products with indications for the treatment of childhood cancers are the vinca alkaloids, which were derived from leaf extracts of the periwinkle plant, and the epipodophyllotoxins, which are semisynthetic derivatives of podophyllotoxin, which was extracted from the roots and rhizomes of the mandrake. The taxanes, which were derived from the yew tree, and the analogs of camptothecin, which was derived from the Chinese tree, *Camptotheca acuminata,* have clinical activity against a variety of adult cancers, but the role of these new agents in treating childhood cancers is still under investigation. As with other natural products, these anticancer drugs have novel and complex chemical structures (Fig. 10-18) and potent biologic properties. [58,824,825,826] and [827] The biotransformation of these drugs is also complex, and the metabolic pathways have only been partially defined.[57,828]

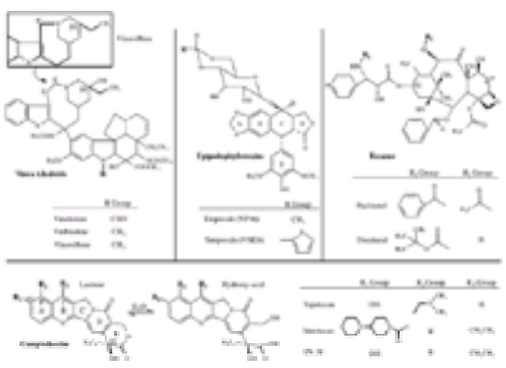


**FIGURE 10-18.** Chemical structures of the plant alkaloids commonly used in the treatment of childhood cancers: the vinca alkaloids, vincristine, vinblastine, and vinorelbine extracted from the periwinkle plant; the epipodophyllotoxins, etoposide and teniposide, synthetic derivatives of the natural product podophyllotoxin, which is derived from the mandrake plant (May apple); the taxanes, paclitaxel and docetaxel, derived from the yew tree; and the camptothecins, topotecan and irinotecan, derived from the stem wood of *Camptotheca acuminata*. Vincristine and vinblastine are identical except for the substituent at the R position, whereas the catharanthine ring of vinorelbine is modified. The asterisks on the taxane structure are hydroxylation sites. The hydroxyl group on the 10-position of SN-38 is the site of glucuronidation.

**Vinca Alkaloids**

The vinca alkaloids, vincristine, vinblastine, and vinorelbine, are structurally similar alkaloids composed of two multiring subunits, vindoline and catharanthine ( Fig. 10-18). Despite their structural similarity, these agents, which act as mitotic inhibitors, have differing clinical and toxicologic properties. The vinca alkaloids exert their cytotoxic effect by binding to tubulin, a dimeric protein that polymerizes to form microtubules. [828,829] The resulting disruption of the intracellular microtubular system

interferes with a number of vital cell functions, including mitosis; maintenance of the cytostructure; movement and transport of solutes, such as neurotransmitters in neuronal axons and hormones and proteins in secretory cells; membrane trafficking and transmission of receptor signals; and transport of p53 to the nucleus. [830,831] The cytotoxic effect of these agents is primarily related to their ability to inhibit mitotic spindle formation, causing metaphase arrest during mitosis. The vinca alkaloids are subject to multidrug resistance, and alterations in the a-and b-tubulin subunits also confer resistance. [830]

Vincristine has a wide spectrum of clinical activity and is currently used in the treatment of ALL, Hodgkin's and non-Hodgkin's lymphomas, rhabdomyosarcoma and other soft tissue sarcomas, Ewing's sarcoma, Wilms' tumor, brain tumors, and neuroblastoma. Vinblastine has been used in the treatment of histiocytosis, testicular cancer, and Hodgkin's disease. Vinorelbine is currently being evaluated in childhood cancers.

***Dosage and Pharmacokinetics***

Vincristine and vinblastine are poorly absorbed if administered orally and are therefore administered intravenously as a bolus injection. Oral vinorelbine is bioavailable, but the resulting plasma concentrations are variable. [832,833] The standard dose for vincristine is 1.0 to 2.0 mg per $m^2$, administered every 1 to 3 weeks. For infants 1 year of age or younger vincristine dose is scaled to body weight (0.03 to 0.05 mg per kg). Many regimens limit the total single dose of vincristine to 2 mg based on reports of increased neurotoxicity at doses above 2 mg, especially on the weekly schedule. However, this practice of capping the dose may underdose some patients, because there is substantial interpatient variation in the plasma pharmacokinetics of vincristine, with a greater than tenfold variation in the AUC.[721,834,835] Escalation of the dose beyond the 2-mg maximum may be well tolerated by some patients. Vinblastine doses range from 3.5 to 6.0 mg per $m^2$, administered in 1- to 3-week cycles. Vinorelbine is administered as a 10-minute infusion at a dose of 30 mg per $m^2$ weekly for up to 6 weeks.

After bolus administration, the vinca alkaloids manifest a rapid initial decline in plasma concentration (initial half-life of 5 to 10 minutes), followed by a prolonged terminal elimination phase with half-life of approximately 12 to 40 hours. [826,828,836,837,838,839,840,841] and [842] The long terminal half-life and the large steady-state volume of distribution (Table 10-6) are consistent with avid and extensive tissue binding that is characteristic of these drugs. Vincristine and vinorelbine clearance is more rapid in children than adults, and adults have a more than twofold longer terminal half-life. [833,836,837] Vincristine disposition in children is highly variable, resulting in a wide interpatient range in drug exposure at a standard dose of 1.5 mg per $m^2$.[836,837,843] Vincristine enters the CSF after intravenous administration, although the CSF concentrations are only 3% to 5% of the corresponding plasma concentrations. [844,845]

Hepatic metabolism and biliary excretion are the principal routes for elimination of the vinca alkaloids. From 70% to 75% of the radioactivity from a radiolabeled dose of vincristine appears in the feces by 72 hours, and slightly more than 10% of the radioactivity is excreted in the urine. [840,846,847] One-half of the radiolabeled material in urine and feces represents metabolites. CYP3A is involved in the metabolism of the vinca alkaloids, [848,849] and [850] and drugs that induce CYP3A4, such as anticonvulsants, and drugs that inhibit CYP3A4, such as azole antifungal agents, can alter the disposition of the vinca alkaloids. [837,851,852,853] and [854] The structures of the various metabolites of the vinca alkaloids are not fully known, but a desacetyl-metabolite of vincristine, vinblastine, and vinorelbine has been identified. [826]

Dosage modifications of the vinca alkaloids are generally recommended in infants and in patients with delayed biliary excretion as evidenced by an elevated direct bilirubin. Infants appear to manifest increased toxicity with standard doses of vincristine based on body surface area. Infants and younger children have a relatively larger ratio of body surface area to weight, and in a randomized crossover study in infants comparing dosing of vincristine based on body surface area (1.5 mg per $m^2$) to dosing by body weight (0.05 mg per kg), the dose calculated from body surface area resulted in greater systemic drug exposure (AUC). [855] Vincristine clearance normalized to body surface area is lower in infants than children, but this difference is not apparent if clearance is normalized to body weight ( Fig. 10-19).[837]

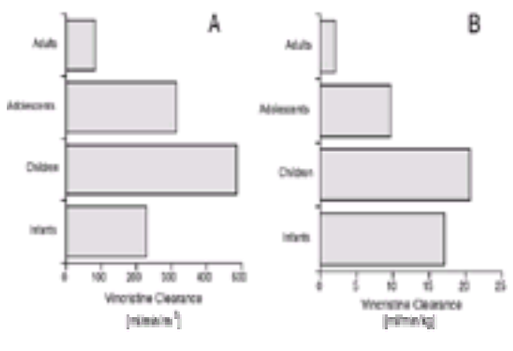



**FIGURE 10-19.** Clearance of vincristine normalized to body surface area **(A)** and body weight **(B) i**n infants (n = 2), children (younger than 10 years, n = 43) and adolescents (n = 9). (Adapted from Crom WR, de Graaf SSN, Synold T, et al. Pharmacokinetics of vincristine in children and adolescents with acute lymphocytic leukemia. J Pediatr 1994; 125:642).

***Toxicity***

Neurotoxicity is the dose-limiting toxicity of vincristine. It is related to the cumulative dose and occurs more commonly on a weekly schedule. Manifestations of the peripheral sensory and motor neuropathy include loss of deep tendon reflexes, neuritic pain (muscular cramping, jaw pain), paresthesias, and wrist and foot drop. Cranial motor nerves may be affected, and autonomic nerve involvement may be responsible for constipation, paralytic ileus, and urinary retention. In most cases, these symptoms are reversible on withdrawal of the drug. Vincristine neurotoxicity can be markedly accentuated in children with Charcot-Marie-Tooth disease. [856] Accidental intrathecal administration of vincristine has been reported and is usually fatal. Other toxicities associated with vincristine include alopecia, inappropriate antidiuretic hormone syndrome, seizures, and orthostatic hypotension. Nausea and vomiting and myelosuppression are rarely encountered. Vincristine can increase the platelet count.

Myelosuppression is the dose-limiting toxic effect of vinblastine and vinorelbine. Vinblastine also frequently causes mucositis. Neurotoxicity with vinblastine is minimal and is less prominent with vinorelbine than vincristine. [830] Vinorelbine causes constipation in 30% of patients. Vinca alkaloids are vesicants; extreme care must be taken to avoid extravasation during their administration. Ulcerations from vinca alkaloid extravasation were prevented in experimental animal model systems with the local injection of hyaluronidase (150 turbidity reducing units) and the application of local warming. [206] Hydrocortisone injection and local cooling increased ulcerations in these studies.

**Epipodophyllotoxins**

Etoposide (VP-16) and teniposide (VM-26) are semisynthetic analogs of the natural product, podophyllotoxin, an antimitotic agent that binds to tubulin. However, the epipodophyllotoxins do not act as microtubule inhibitors. [857,858] Instead, these glycosidic derivatives of podophyllotoxin ( Fig. 10-18) exert their antitumor effect through stabilization of the normally transient covalent intermediates formed between the DNA substrate and topoisomerase II, leading to single- and double-strand DNA breaks.[56,859,860,861,862] and [863] Resistance to epipodophyllotoxins can result from increased activity of the P-gp and related membrane efflux pump responsible for multidrug resistance, resulting in decreased intracellular drug accumulation, and from altered topoisomerase II activity (lower enzyme levels, phosphorylation of the enzyme, or mutations leading to decreased affinity for the drug), leading to a reduction in the formation of drug-induced cleavable complexes. [857]

There are no major differences in the antitumor spectra of these two drugs. Activity has been observed against the acute leukemias, Hodgkin's and non-Hodgkin's lymphomas, neuroblastoma, rhabdomyosarcoma and other soft tissue sarcomas, Ewing's sarcoma, germ cell tumors, and brain tumors.[864,865,866] and [867]

***Dosage and Toxicity***

Because the solubility of the epipodophyllotoxins in water is poor, both are supplied in nonaqueous formulations. Etoposide is formulated in polysorbate 80,

polyethylene glycol, and alcohol, and teniposide is formulated in Cremaphor EL, alcohol, and dimethylacetamide. Before intravenous administration, these agents are diluted in 5.0% dextrose in water or 0.9% saline to a concentration of less than 0.4 mg per mL and infused over 30 to 60 minutes to avoid the hypotension associated with rapid injections. Etoposide phosphate is a water-soluble prodrug of etoposide that overcomes the formulation difficulties of the parent drug. [868] Etoposide phosphate is rapidly converted to etoposide *in vivo* by plasma phosphatases and has a toxicity profile, maximum tolerated dose, and pharmacokinetic profile similar to that of etoposide.[869,870]

Etoposide is usually administered on a daily schedule for 3 to 5 days at a dose of 60 to 120 mg per $m^2$ per day. Teniposide is administered at a dose of 70 to 180 mg per $m^2$ daily for 3 days. Both agents have also been administered on a single high-dose schedule (up to 800 mg per $m^2$ of etoposide and up to 1,000 mg per $m^2$ of teniposide), and etoposide (2,400 mg per $m^2$) has been incorporated into bone marrow transplant preparative regimens. A chronic oral low-dose (50 mg per $m^2$ per day) schedule of etoposide has also being studied. [871,872]

Etoposide antitumor activity is dose- and schedule-dependent.[871,873,874] In adults with small cell lung cancers, the response rate in patients treated on a daily-for-5-days schedule is significantly higher than in patients treated with same total dose infused over 24 hours. [875] The chronic oral dosing schedule is also highly active in a variety of adult cancers.[876,877] However, a comparative trial of cisplatin in combination with either 21 days of oral etoposide at 50 mg per $m^2$ per day or 3 days of intravenous etoposide at 130 mg per $m^2$ per day in adults with lung cancer failed to demonstrate a survival advantage for this 21-day oral dosing schedule. [878]

The primary dose-limiting toxicity of the epipodophyllotoxins is myelosuppression. Other toxicities include alopecia, nausea, vomiting, phlebitis, mild peripheral neuropathy, hepatocellular enzyme elevations, and mucositis. Arrhythmias are relatively rare. Diarrhea was the dose-limiting toxicity in children treated with etoposide on the chronic oral dosing schedule, but myelosuppression and mucositis were also prominent toxicities. [879] Non–dose-limiting hypersensitivity reactions, which are characterized by urticaria, flushing, rash, and angioedema, are common and related to the cumulative dose of etoposide or teniposide. [880] Severe hypersensitivity reactions, such as bronchospasm and anaphylaxis, are less common and occur less frequently with etoposide than with teniposide. [881] A severe skin rash has also been reported with high-dose teniposide. [882]

A distinctive form of secondary acute leukemia, characterized by a short latency period (median time to presentation, 30 months), chromosomal translocations involving chromosome band 11q23, and M4 or M5 FAB morphologic subtype (monocytic or myelomonocytic), has been reported with alarming frequency in epipodophyllotoxin-treated patients.[883,884,885 and 886] The cumulative risk of developing this form of secondary leukemia has been estimated to be 5% to 12% in children with ALL treated with high cumulative doses of epipodophyllotoxins on a weekly or twice-weekly schedule. [886] In contrast, the incidence of this form of secondary acute nonlymphoblastic leukemia in survivors of germ cell cancers who were treated with etoposide is less than 1%. The 6-year cumulative incidence of secondary leukemia and myelodysplastic syndrome in patients who were treated on 12 pediatric cooperative group clinical trials was 3.3%, 0.7%, and 2.2% for cumulative etoposide doses of less than 1.5 g per $m^2$, 1.50 to 2.99 g per $m^2$, and greater than or equal to 3 g per $m^2$, respectively. Thus, epipodophyllotoxin cumulative dose does not appear to be a risk factor for development of secondary leukemia. [887]

***Pharmacokinetics and Drug Interactions***

The disposition of the epipodophyllotoxins is characterized by a significant degree of intrapatient and interpatient variability. [888,889] The bioavailability of oral etoposide is approximately 50% at doses of 200 mg per $m^2$ or less, but it ranges from 10% to 80%,[890,891] and there is considerable dose-to-dose variation within each patient.[892,893] Bioavailability is also nonlinear. At higher doses (greater than 200 mg per $m^2$), the fraction of the dose absorbed decreases.[894,895] Because oral absorption is erratic and dose-dependent and this route of administration has been associated with increased toxicity, the clinical usefulness of oral administration of standard doses of etoposide has been limited. However, the more efficient absorption of lower doses of etoposide (bioavailability, 70%) suggests that the chronic oral low-dose schedule may circumvent some of these limitations.[896,897] The mean bioavailability of etoposide from oral etoposide phosphate is 76% (range, 37% to 144%),[898] and the mean bioavailability of teniposide is 40% (range, 20% to 70%). [899] The absorption of teniposide also appears to decrease as the dose is increased.

The epipodophyllotoxins are extensively metabolized, although specific details of the metabolic pathways have not been fully elucidated. Some of these metabolites retain cytotoxic activity.[888,900] Metabolites identified in urine include the hydroxy acid derivatives [901] and glucuronide and sulfate conjugates.[888,902] The epipodophyllotoxins also undergo CYP3A4 mediated O-demethylation to the active catechol form, which can be oxidized to a reactive quinone. [903] Renal clearance accounts for 30% to 40% of the total systemic clearance of etoposide, but less than 10% of teniposide clearance. [892,899,904,905,906 and 907] This difference probably reflects the difference in the degree of protein binding of the two drugs (Table 10-6). Biliary excretion is not a major route of elimination for etoposide, accounting for less than 10% of total drug elimination in most studies. [902] Penetration of the epipodophyllotoxins into the CSF is limited, [865,905,908] but the concentrations achieved may be cytotoxic.[909]

The clearance of the epipodophyllotoxins is highly variable. For etoposide, the median clearance in children is 26 mL per minute per $m^2$, and the range is 14 to 54 mL per minute per $m^2$.[908] For teniposide, the median clearance is 13 mL per minute per $m^2$, and the range is 4 to 22 mL per minute per $m^2$.[910] The pharmacokinetic parameters are independent of the dose for doses up to 3,000 mg per $m^2$.[905,908,911] The clearance of etoposide is also age independent. In infants 3 to 12 months of age, the median clearance was 19 mL per minute per $m^2$, and in children older than 1 year of age, the median clearance was 18 mL per minute per $m^2$.[912] Therefore, no special dosing guidelines are required for treating infants, and all patients should receive a dose calculated from body surface area.

The pharmacokinetics of etoposide have been evaluated in patients with hepatic and renal dysfunction. [908,913,914,915 and 916] Etoposide clearance was significantly delayed and the terminal half-life prolonged in patients with renal insufficiency, putting them at higher risk for toxic reactions. Overall, correlation was good between creatinine clearance and etoposide clearance in these studies, suggesting that etoposide dose modifications should be based on the creatinine clearance. Etoposide clearance was not delayed in patients with abnormal hepatic function, indicating that dose adjustments are not necessary in these patients. The protein binding of etoposide is highly variable in cancer patients (range, 76% to 97%), and the degree of binding is correlated with the serum albumin level. [917,918] Patients with low serum albumin experience more severe hematologic toxicity from etoposide, presumably because of higher free-drug concentrations. [916,919] A 30% to 40% dosage reduction may be indicated in these patients. The fraction of etoposide bound to protein is higher in pediatric cancer patients than in adults with cancer. [920]

The wide interpatient variation in plasma concentrations of etoposide and teniposide has prompted investigators to evaluate the relation between plasma drug levels and measures of toxicity and response and to develop dosing methods that incorporate dose adjustments based on the plasma drug concentration. [921,922,923 and 924] In children treated with escalating doses of teniposide by continuous infusion, there was a fivefold variation in steady-state plasma teniposide concentration at a given dose, and the plasma drug concentration was a better predictor of response than the dose. [923] This led to trials in which the dose rate of teniposide was individually adjusted to normalize the plasma teniposide steady-state concentration to 15 μM for the 72-hour infusion schedule [922] or the total AUC to 1,500 μM • hour for the daily-for-3-days schedule. [910] Eliminating the wide variation in drug exposure by adjusting the dose to achieve a target plasma concentration allows increased dose intensity without increasing the risk of acute toxicity. [910] Dose adjustment based on the AUC after the first dose of etoposide is precise in achieving a target AUC for subsequent doses in children.[924] Attempts to apply therapeutic drug monitoring to low-dose oral etoposide regimens have had limited success, because of the marked intrapatient variability in drug absorption and disposition. Because of the variability in the extent of protein binding of etoposide, dosage adjustments based on the nonprotein-bound (free) fraction of etoposide may prove more successful than total drug concentration. [925,926]

Cyclosporine, a modulator of the P-gp, diminishes the renal and nonrenal elimination of etoposide and teniposide, resulting in an increase in plasma exposure (AUC) to the drugs and an increase in clinical toxicity. [927,928 and 929] The concomitant administration of anticonvulsants with etoposide and teniposide in children results in a two- to threefold increase in clearance and a proportional decrease in systemic drug exposure, which could reduce the drugs' efficacy. [930,931] The enhanced clearance is presumably the result of induction of hepatic metabolism.

**Taxanes**

The taxanes, paclitaxel and docetaxel, are complex diterpenes (Fig. 10-18) that exert their cytotoxic effect by interfering with microtubule function. However, unlike the vinca alkaloids, the taxanes increase microtubule stability by preventing microtubule depolymerization, which results in tubulin bundling. [932,933 and 934] Taxane-induced cytoskeletal changes lead to cell cycle arrest in the $G_2$ (premitotic) and M (mitotic) phases and cell death by apoptosis. Because paclitaxel arrests cells in the $G_2$/M phase, the most radiosensitive phase of the cell cycle, it is also a potent radiosensitizer. [933,934] Resistance to taxanes *in vitro* has been ascribed to the development of

altered tubulin subunits with impaired ability to polymerize, to the presence of the P-gp drug-efflux pump responsible for multidrug resistance, and to elevated levels of Raf-1 kinase, which can suppress paclitaxel-induced apoptosis.[932,935,936]

Although paclitaxel and docetaxel have a broad spectrum of antitumor activity in adult cancers, paclitaxel does not appear to be active against common childhood cancers as a single agent.[937] Docetaxel is still under evaluation. Paclitaxel is insoluble in water and is formulated in Cremophor EL and ethanol. This vehicle has been implicated in the hypersensitivity reactions from paclitaxel. Because this formulation of paclitaxel leaches plasticizers (phthalate) from polyvinylchloride intravenous infusion bags, it must be mixed and stored in glass, polypropylene, or polyolefin containers and administered through polyethylene-lined infusion sets. Docetaxel is formulated in polysorbate 80 and ethanol.

Paclitaxel has been administered as a 1-, 3-, 24-, or 96-hour intravenous infusion. The standard adult dose of paclitaxel is 135 or 175 mg per $m^2$ infused over 3 hours, although doses of up to 250 mg per $m^2$ are tolerable. Longer infusions appear to be more myelosuppressive.[938,939] The recommended dose of paclitaxel administered as a 24-hour infusion in children is 350 mg per $m^2$.[940] Docetaxel is administered as 1-hour infusion every 3 weeks at a dose of 100 to 125 mg per $m^2$, but higher doses may be tolerable with Filgrastim support.[941,942]

Paclitaxel pharmacokinetics are dose dependent and probably schedule dependent, and complex pharmacokinetic models incorporating capacity-limited elimination and capacity-limited distribution have been devised to describe the disposition of the drug.[943,944] Paclitaxel is extensively tissue bound, accounting for its large volume of distribution. Hepatic metabolism (hydroxylation) by CYP2C8 at the C-6 position on the ring and by CYP3A4 on the C-13 side chain ( Fig. 10-18) followed by biliary excretion are the primary routes of paclitaxel elimination (80% of the dose is recovered as parent drug or metabolites in feces).[945,946,947 and 948] Docetaxel also undergoes hepatic metabolism by CY3A4 and biliary excretion.[949,950] Drugs or xenobiotics that induce CYP450 enzymes, such as the anticonvulsants, enhance paclitaxel clearance,[951,952] and CYP3A4 inhibitors, such as the imidazole antifungal agents, reduce paclitaxel clearance.[945] Patients with hepatic tumor involvement or biochemical evidence of liver dysfunction (e.g., elevated bilirubin or transaminases) are at increased risk for paclitaxel toxicity, presumably because of delayed paclitaxel clearance.[953,954] Dosage reductions are recommended for these patients.[953] Renal excretion accounts for only 5% of total drug clearance.[945,955]

Myelosuppression is the primary dose-limiting toxicity of paclitaxel.[830,956] Neurotoxicity is also prominent in children and is characterized by a stocking-glove peripheral neuropathy (paresthesias, diffuse myalgias, and loss of fine motor control) and seizures.[940] Acute encephalopathy and irreversible coma are also associated with paclitaxel.[957,958] Ethanol in the formulation can cause toxicity if high doses of paclitaxel are infused over a short period.[959,960] The incidence of acute hypersensitivity reactions (hypotension, urticaria, and bronchospasm) occurring within minutes of the start of the infusion[961] has been reduced by administering paclitaxel as a more prolonged infusion and by premedicating patients with corticosteroids and antihistamines (e.g., Benadryl and ranitidine). Cardiac arrhythmias (e.g., bradycardia and atrioventricular conduction disturbances), alopecia, mucositis, radiation-recall dermatitis, pneumonitis, and phlebitis at the injection site are also caused by paclitaxel.[830,934,962]

Docetaxel produces neutropenia without significant thrombocytopenia. Other toxicities include malaise, myalgias, skin rashes (including palmar-plantar erythrodysesthesia), nausea and vomiting, mucositis, diarrhea, alopecia, interstitial pneumonitis, and transient elevations of serum transaminases.[941,942,963] Neurotoxicity is less prominent with docetaxel, but fluid retention associated with weight gain, edema, and in some cases scleroderma-like skin changes, is a cumulative toxicity that occurs in 20% of patients.[941,964,965] Hypersensitivity reactions, rashes, and fluid retention may be ameliorated by premedication with an antihistamine and corticosteroid.

### Camptothecins

Topotecan and irinotecan are semisynthetic, water-soluble camptothecin analogs ( Fig. 10-18) that produce DNA strand breaks by forming a ternary complex with DNA and topoisomerase I.[58,825,827] In aqueous solutions, the camptothecins exist in an equilibrium between the active lactone form and the relatively inactive hydroxy-acid form, which results from reversible hydrolysis of the E-ring. The inactive form predominates at physiologic pH, although the ratio of lactone:hydroxy acid varies for topotecan (10%), irinotecan (25% to 30%) and its active metabolite SN-38 (50% to 65%).[825,827,966] Decreased intracellular levels of topoisomerase I, alterations in the affinity of topoisomerase I for the camptothecin analogs, and expression of membrane-associated drug efflux pumps (P-gp) are mechanisms of resistance to topotecan and irinotecan.[967,968,969,970,971,972,973 and 974] However, the camptothecin analogs are poor substrates for the P-gp.[975]

#### *Topotecan*

Topotecan appears to be active against neuroblastoma and rhabdomyosarcoma,[976] but its role in treating these and other childhood cancers is still being defined. It is usually administered daily for 5 days at a dose of 1.4 mg per $m^2$ per day or 2.0 mg per $m^2$ per day with filgrastim.[977] Continuous infusion schedules of 1 to 21 days' duration have also been studied. Topotecan dose must be substantially reduced when administered in combination with cisplatin or cyclophosphamide because of enhanced hematologic toxicity.[978,979] Myelosuppression is the most common topotecan toxicity. Diarrhea becomes dose limiting with more protracted schedules or with oral dosing.[980] Other toxicities associated with topotecan include nausea and vomiting, alopecia, mucositis, elevated hepatic transaminases, and rash.[977,981,982]

The bioavailability of oral topotecan in children is approximately 30%, but there is marked interpatient variability in absorption.[983] With intravenous administration, the clearance of the lactone form of topotecan is also highly variable.[981,984] The terminal half-life of topotecan is 3 to 5 hours, and renal excretion is the primary route of elimination (60% to 70% of total dose).[981,985,986] Impaired renal function decreases topotecan clearance, necessitating a dosage reduction.[987] N-demethylation is a minor metabolic pathway,[988] and mild to moderate hepatic dysfunction does not appear to impact on drug disposition.[989] Topotecan penetrates into the CSF better than other topoisomerase I inhibitors.[990,991]

#### *Irinotecan*

Irinotecan is a prodrug that is converted by carboxylesterase in the liver and intestinal tract to the active metabolite, 7-ethyl-10-hydroxy camptothecin (SN-38), which is 100- to 1,000-fold more potent than irinotecan. In adults, irinotecan is administered as a 90-minute intravenous infusion weekly for 4 weeks at a dose of 125 mg per $m^2$ per day. Several dosing schedules are being evaluated for childhood cancers.[992,993 and 994] Myelosuppression and diarrhea are the most common toxicities in children and adults. Diarrhea, diaphoresis, and abdominal cramping that are associated with the drug infusion[995] are responsive to atropine, and delayed diarrhea is responsive to loperamide.[996] Other irinotecan toxicities include nausea and vomiting, transient elevations of hepatic transaminases, asthenia, alopecia, malaise, and electrolyte abnormalities.[993,994,997]

Oral irinotecan is rapidly absorbed and more efficiently converted to SN-38 due to first-pass metabolism, but plasma drug and metabolite concentrations are highly variable.[998] With intravenous administration, conversion of irinotecan to SN-38 is inefficient (less than 10% of the dose).[966,999] Oxidation of the dipiperidine side-chain by CYP3A subfamily enzymes also yields two minor metabolites (7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin [APC] and 7-ethyl-10-(4-amino-1-piperidino)carbonyloxycamptothecin [NPC]).[1000] Induction of these CYP3A catabolic pathways by anticonvulsants can enhances the clearance of irinotecan and reduce the production of SN-38.[1001,1002] Irinotecan and its metabolites are eliminated primarily by biliary excretion.[1000] Renal excretion of the parent drug accounts for 15% to 25% of the dose.[966]

SN-38 is conjugated to SN-38 glucuronide (SN-38G) by hepatic uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1), the enzyme responsible for bilirubin conjugation.[1003] Patients who have partial UGT1A1 deficiency (Gilbert's or Crigler-Najjar syndromes) or who are receiving drugs that inhibit UGTA1A, such as valproic acid, are at risk for increased drug-related toxicity.[1004,1005] SN-38G is secreted into the bile and deconjugated to SN-38 by beta-glucuronidase in the gut. This intraluminal formation of SN-38 may be responsible for the delayed diarrhea from irinotecan.[1005] The product of the irinotecan AUC and the ratio of SN-38 and SN-38G AUCs (biliary index) is higher in patients with more severe diarrhea.[1006]

## MISCELLANEOUS AGENTS

### Corticosteroids

Although they are not generally thought of as anticancer drugs because of the diversity of their other clinical uses, the corticosteroids (prednisone, prednisolone, and dexamethasone) play a significant role in the treatment of ALL, lymphoma, and Hodgkin's disease and have been incorporated into treatment regimens for the histiocytoses and brain tumors. They are also useful in managing some of the complications of cancer, including hypercalcemia, increased intracranial pressure, anorexia, and chemotherapy-induced nausea and vomiting.

Glucocorticoids induce apoptosis by binding to intracellular glucocorticoid receptors. [1007] The receptor-glucocorticoid complex translocates to the nucleus, dimerizes, and binds to specific DNA response elements. This causes modulation of expression of many genes. Continuous saturation of the receptor by the steroid for many hours to days is needed to induce apoptosis in sensitive cell lines, and in children with ALL, thrice daily administration is more effective than intermittent schedules.[1007] Glucocorticoid receptor content on leukemic blasts and the duration of receptor occupancy appear to be the critical determinants of response to corticosteroid therapy *in vitro* and *in vivo*. Loss of or defect in the glucocorticoid receptor can lead to drug resistance *in vitro*.[1008],[1009] and [1010] Children with ALL and low levels of glucocorticoid receptor on their lymphoblasts have a poor prognosis when treated on corticosteroid-based regimens.

The chemical structures of the most commonly used synthetic analogs of cortisol, prednisone, prednisolone, and dexamethasone, are shown in Figure 10-20. The addition of the 1,2-double bond in prednisolone and dexamethasone increases the glucocorticoid and antiinflammatory potency fourfold and decreases mineralocorticoid activity. Further addition of the fluorine at position 9 in dexamethasone enhances the activity another fivefold. Prednisone is an inactive prodrug analogous to cortisone and requires chemical reduction of the ketone group at position 11 to a hydroxyl group, yielding prednisolone. This activation occurs in the liver.[1011],[1012] Prednisolone and dexamethasone are eliminated by the catabolic enzymes that inactivate cortisol by reduction of the 4,5-double bond (hepatic and extrahepatic), hydroxylation at the 6-position (hepatic), or reduction of the 3-ketone to a hydroxyl group followed by conjugation with a sulfate or glucuronide (hepatic).[1012]

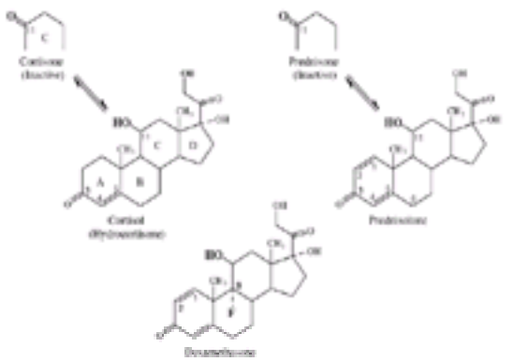


**FIGURE 10-20.** Chemical structures of the naturally occurring and synthetic corticosteroids commonly used in treating childhood cancers. Reduction of the keto group (cortisone and prednisone) to a hydroxyl group (cortisol and prednisolone) at the 11 position is necessary for activity. Addition of the 1,2-double bond (prednisolone and dexamethasone) and the fluorine group at the 9 position (dexamethasone) increases glucocorticoid activity.

### *Pharmacokinetics*

The absorption of orally administered prednisone, prednisolone, and dexamethasone is almost complete (greater than 80%). [1011],[1012] and [1013] Prednisone is rapidly converted to prednisolone, which is the predominant form in plasma after an oral dose of prednisone. [1011],[1012] In children, variable absorption of prednisone and prednisolone has been reported.[1013],[1014] The elimination half-lives are 2.5 hours for prednisolone and 4 hours for dexamethasone, reflecting differences in the rate of catabolism.[1014],[1015],[1016],[1017] and [1018] Hepatic metabolism is the primary route of elimination; renal clearance accounts for 10% or less of total clearance. [1019],[1020] The clearance of prednisolone is dose dependent and increases with increasing dose, because of concentration-dependent binding of prednisolone to plasma proteins.[1012],[1020],[1021] At low concentrations, prednisolone, like cortisol, is more than 95% bound to transcortin, but this specific carrier protein is saturated at higher prednisolone concentrations, so that the relative amount of free drug available for metabolic degradation increases.

Dexamethasone is not bound to transcortin, and the degree of protein binding is concentration independent. [1022] The lower rate of meningeal relapse in children treated with dexamethasone compared with prednisone may be explained by this difference in protein binding. [1023],[1024] and [1025] The concentration of prednisolone and dexamethasone in the CSF is equivalent to the free-drug concentration in plasma, and because prednisolone, unlike dexamethasone, is tightly and extensively bound to transcortin at low concentrations, its free plasma and CSF concentrations are lower at equipotent doses. Diurnal variation in plasma concentrations of prednisolone has also been observed with oral dosing. The drug concentrations are higher in the morning than in the evening after equal doses. [1021]

The capacity to activate prednisone to prednisolone is not impaired in patients with severe hepatic dysfunction, and prednisolone levels are elevated in this group, because of delayed catabolism. Unbound prednisolone concentration is also elevated in patients with severe renal dysfunction. [1012]

### *Toxicity*

The corticosteroids have some effect on almost every organ and tissue in the body, and the side effects of these agents are protean. Significant common toxicities include increased appetite, centripetal obesity, immunosuppression, myopathy, osteoporosis, avascular necrosis of the hip, peptic ulceration, pancreatitis, psychiatric disorders, cataracts, hypertension, precipitation of diabetes, growth failure, amenorrhea, impaired wound healing, and atrophy of subcutaneous tissue. [1008],[1022] Osteonecrosis of weightbearing joints occurs in children with high-risk ALL, and risk factors included age older than 10 years, female gender, and treatment with two rather than one 21-day course of dexamethasone.[1026]

### *Drug Interactions*

Ketoconazole interferes with the elimination of non–protein-bound prednisolone by inhibiting the catabolic enzyme, 6-b-hydroxylase, leading to a 50% increase in the AUC of unbound prednisolone in patients taking concurrent oral ketoconazole. [1027] Estrogen-containing oral contraceptives increase transcortin and lower free prednisolone concentrations.[1012] Drugs such as phenytoin, rifampicin, carbamazepam and barbiturates induce hepatic microsomal enzymes that catabolize prednisolone and result in enhanced prednisolone clearance. [1012]

## Asparaginase

Asparaginase is a bacterial enzyme that provides specific nutritional therapy for ALL and lymphomas. This enzyme rapidly depletes the circulating pool of asparagine by catalyzing the conversion of this amino acid to aspartic acid and ammonia. In most tissues, asparagine is synthesized from aspartic acid and glutamine by the enzyme asparagine synthase, and normal tissues can respond to asparagine depletion by up-regulation of this enzyme. In contrast, sensitive lymphoid cancers do not up-regulate asparagine synthase and, therefore, depend on exogenous circulating asparagine for protein synthesis. [1028] Asparaginase-resistant lymphoid tumor cells often have high levels of asparagine synthase, rendering them capable of synthesizing their own asparagine. [1028] Thus, asparaginase has a selective antileukemic effect.[1028],[1029]

The native (unmodified) forms of asparaginase are derived from *Escherichia coli* or *Erwinia carotovora* and are administered intravenously or intramuscularly at doses of 6,000 to 25,000 IU per $m^2$ on an intermittent schedule (usually three times each week). The intramuscular route is more commonly used in children in the United States, because of a lower risk of severe allergic reactions. [1030] Conjugation of polyethylene glycol to *E. coli* asparaginase (PEG-asparaginase) lowers the immunogenicity of this foreign protein and prolongs the drug's half-life, allowing for less frequent administration (2,500 IU per $m^2$ every 2 to 4 weeks).[1031] The $k_m$ of *E. coli* asparaginase for asparagine is 10 µM, which is approximately tenfold higher than the minimal concentration of the amino acid required *in vitro* to support cell

growth.[1028] The continual production and release of asparagine by normal tissues into the blood stream requires plasma asparaginase activity exceeding 0.1 IU per mL to suppress the concentration of asparagine below the critical level of 1 to 3 μM. [1032,1033] Doses of *E. coli* asparaginase at 2,500 IU per $m^2$ appear to be sufficient to deplete serum asparagine,[1034] whereas higher doses and more frequent administration of *Erwinia* asparaginase may be required.[1035]

In a randomized trial in standard-risk ALL, asparaginase plasma levels were more prolonged with a single dose of PEG-asparaginase than with nine to 12 doses of native *E. coli* asparaginase.[1036] Toxicity of the native and pegylated enzymes was similar, and the incidence of allergic reactions and silent antibodies was very low with both preparation. More prolonged asparaginase treatment regimens improve disease outcome but are associated with a higher incidence of allergic reactions.[1037,1038]

***Pharmacokinetics***

Parenteral administration of asparaginase is required because of denaturation and peptidase digestion within the intestinal tract. Peak plasma concentrations of the enzyme are dose related. Daily administration results in significant accumulation of asparaginase. [1039] Peak concentrations with intramuscular injection are approximately one-half those achieved with intravenous dosing. The time to peak concentration (rate of absorption) after intramuscular injection is 24 to 48 hours for *E. coli* asparaginase, less than 24 hours for *Erwinia* asparaginase, and 72 to 96 hours for PEG-asparaginase.[1040] The volume of distribution for asparaginase approximates plasma volume, and the rate of elimination is slow. The half-lives for *E. coli* asparaginase, *Erwinia* asparaginase, and PEG-asparaginase are 24 to 36 hours, 10 to 15 hours, and 5 to 7 days, respectively (Fig. 10-21).[1040]

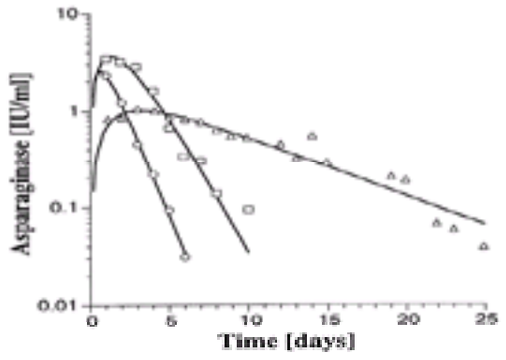



**FIGURE 10-21.** Disposition of serum asparaginase activity in ten patients treated intramuscularly with 25,000 IU per $m^2$ of native *Escherichia coli* asparaginase (*open squares*), ten patients treated intramuscularly with 25,000 IU per $m^2$ of native *Erwinia* asparaginase (○), and ten patients treated intramuscularly with 2,500 IU per $m^2$ of polyethylene glycol polyethylene glycol-asparaginase ( △). Points represent the mean. (Adapted from Asselin BL, Whitin JC, Coppola DJ, et al. Comparative pharmacokinetic studies of three asparaginase preparations. J Clin Oncol 1993;11:1780).

Plasma concentrations of asparagine fall to undetectable levels within 24 hours of a dose of asparaginase. The duration of depletion of circulating asparagine is related to the rate of asparaginase elimination; consequently it is shorter with *Erwinia* asparaginase than with native *E. coli* asparaginase.[1034] PEG-asparaginase depletes serum asparagine for at least 14 days.[1041,1042] Even though asparaginase distributes primarily within the intravascular space, its effects are more wide reaching. For example, asparaginase cannot be detected in the CSF after systemic administration, but CSF levels of asparagine are depleted for long periods. [1043,1044] and [1045]

Patients who develop antibodies to asparaginase have a rapid fall in the plasma concentrations of the native enzyme, indicating that the antibody interferes with the therapeutic effects of asparaginase. [1029,1046] PEG-asparaginase elimination is more rapid in one-third of patients who have previously experienced hypersensitivity reactions to native *E. coli* asparaginase.[1040,1047] Consequently, weekly dosing of PEG-asparaginase is recommended in children previously treated with native asparaginase.[1040,1048] In children with relapsed ALL, weekly administration of PEG-asparaginase resulted in a higher remission induction rate than biweekly dosing. [1048]

***Toxicity***

The principal side effects of asparaginase are related to sensitization to a bacterial protein or decreased protein synthesis. [1029,1033] Allergic reactions range from local erythema and swelling at the injection site to urticaria, laryngeal edema, bronchospasm, or anaphylaxis. Diphenhydramine, epinephrine, and other resuscitative measures must be available when administering this agent, even for the initial dose. The overall incidence of hypersensitivity reactions in children is 16% to 33% with native *E. coli* asparaginase.[1029] PEG-asparaginase is less immunogenic than the native forms, but acute allergic reactions can occur with repeated administration. The incidence of hypersensitivity reactions is lower (10%) in patients receiving combination chemotherapy than in those receiving *E. coli* asparaginase as a single agent (40%), presumably because of the immunosuppressive effects of the other drugs in the regimen. [1049] *E. coli* and *Erwinia* asparaginase are minimally cross-reactive, so those patients experiencing hypersensitivity reactions to one can be safely be switched to the other. [1050] PEG-asparaginase has also been administered safely to patients who were hypersensitive to the native *E. coli* enzyme.[1031,1048]

Coagulopathies resulting from deficiencies or imbalances in coagulation factors (fibrinogen, II, V, VII, VIII-X, antithrombin III, and protein C) can lead to clotting and hemorrhagic complications, including stroke. The thromboembolic events reflect a decreased capacity to inhibit thrombin, resulting from the acquired antithrombin III deficiency.[1051] Decreased serum albumin, insulin, and lipoproteins are also associated with asparaginase therapy. Other toxicities include an encephalopathy characterized by somnolence, disorientation, seizures, and coma, which have been related to hyperammonemia in some patients; acute pancreatitis, which can progress to hemorrhagic pancreatitis; and hepatotoxicity characterized by hyperbilirubinemia and elevated serum transaminases. [1029,1033] Myelosuppression and gastrointestinal toxicity (with the exception of nausea and vomiting) are usually not observed.

***Drug Interactions***

Asparaginase can rescue patients from the toxic effects of methotrexate and cytarabine. The sequential combinations of methotrexate or cytarabine followed by asparaginase have proven effective in consolidation therapy of ALL and in the treatment of children with relapsed leukemia. [1029,1033] The rescue effect is usually attributed to a decrease in protein synthesis in normal tissue. Antagonism has been observed if asparaginase is administered before these antimetabolites. [1052]

**Protein Kinase Inhibitors**

Protein kinases play a key regulatory role in cellular growth, differentiation, and apoptosis. The genes encoding these enzymes are aberrantly activated by a variety of mechanisms, such as translocations and point mutations, in human cancers. Although transforming mutations to tyrosine kinase genes are rare in childhood cancers, the *BCR-ABL* fusion protein expressed by the Philadelphia chromosome (Ph) in CML and 3% to 5% of children with ALL is clinically significant, because it confers a poor prognosis. A number of small molecule inhibitors of these protein kinases are in clinical development. [1053] STI-571 is a 2-phenylpyrimidine derivative that selectively inhibits the *BCR-ABL* fusion protein and selectively induces apoptosis in Ph-positive ($Ph^+$) CML cells by blocking *BCR-ABL*-initiated signaling pathways.[1054] ABL-initiated signaling pathways have recently been approved by the FDA. Daily oral dosing of STI-571 is very well tolerated and has produced hematologic remissions in most patients with $Ph^+$ CML. Disappearance of $Ph^+$ cells from the bone marrow has been observed in a few patients after treatment with this agent.[1055]

## CENTRAL NERVOUS SYSTEM PHARMACOLOGY OF ANTICANCER DRUGS

The penetration of the anticancer drugs into the CNS is relevant to the treatment of childhood cancers, because primary and metastatic tumors of the brain or meninges are common in children and because anticancer drugs are associated with acute and chronic neurotoxicity. The degree of drug penetration across the

blood–brain barrier is determined by the physicochemical properties of the drug, such as lipophilicity, molecular size, and degree of ionization, and the free (non–protein-bound) drug concentration in plasma. [1056,1057] and [1058] Most anticancer drugs penetrate poorly into the CSF, which is also used as a surrogate for blood–brain barrier penetration ( Table 10-6). The inability to achieve adequate antileukemic drug concentrations in the CSF probably accounts for the high rate of leptomeningeal relapse in children with ALL after the introduction of effective systemic therapy. Strategies used to circumvent limited penetration into the CNS are listed in Table 10-8. Regional drug administration, such as intrathecal, intraarterial, and interstitial implants, is the most commonly used approach. [1056]

| Strategy | Examples |
|---|---|
| High-dose systemic chemotherapy | High-dose methotrexate, cytarabine |
| Identifying drugs that penetrate the blood-brain barrier (cross into cerebrospinal fluid) | Thiotepa, topotecan |
| Disruption of the blood–brain barrier | Osmotic disruption with mannitol |
| Regional drug administration | |
| Intrathecal injection | Methotrexate, cytarabine |
| Intraarterial injection | Cisplatin |
| Interstitial therapy | Gliadel |

**TABLE 10-8. TREATMENT STRATEGIES TO CIRCUMVENT THE BLOOD–BRAIN BARRIER**

### Intrathecal Chemotherapy

Poor penetration of systemically administered anticancer drugs into the CSF can be circumvented by direct injection of the agents into the CSF. Intrathecally injected chemotherapy (e.g., methotrexate and cytarabine) is highly effective as primary or preventive therapy for meningeal leukemia and lymphoma. As a form of regional chemotherapy, intrathecal administration has the advantage of delivering very high drug concentrations to the CSF and meninges with low doses and therefore with minimal systemic toxicity.[1056,1059] However, there are disadvantages to intralumbar administration. Repeated lumbar punctures are painful, inconvenient, and technically challenging. In 10% of intralumbar injections, the drug is not delivered into the subarachnoid space but is instead injected or leaks into the subdural or epidural space.[1060] Because of the slow circulation of the CSF, distribution of drugs within the subarachnoid space, specifically to the ventricles, is not uniform. Ventricular methotrexate concentrations after an intralumbar dose are highly variable and are less than 10% of ventricular concentrations after direct intraventricular injection.[1061] The depth of penetration of effective drug concentrations into the brain parenchyma is limited to a few millimeters for the commonly used intrathecal agents because of the rapid diffusion of drug into capillaries within the highly vascular brain tissue. As a result, intrathecal therapy is not likely to be effective for parenchymal brain tumors.[1062] Intrathecal therapy is also associated with unique toxicities, such as chemical arachnoiditis.

The distribution of drug from the lumbar sac to the ventricles can be significantly improved by positioning the patient prone for 60 minutes after intralumbar injection. In animals, ventricular methotrexate concentrations after intralumbar drug administration were more than 20-fold higher when they were placed in the prone position compared to keeping them upright.[1063] Many of the problems associated with intralumbar injection can also be overcome with direct intraventricular administration. This approach entails the surgical placement of a catheter into the lateral ventricle. The catheter is then attached to a subcutaneously implanted reservoir for access.[1064] Administration of drugs directly into the ventricle via this reservoir is more convenient and less painful and allows for more frequent injections. Intraventricular therapy ensures that the drug is delivered to the subarachnoid space and results in better drug distribution throughout the CSF. [1061] Better drug distribution may account for the improved therapeutic results from intraventricular therapy compared with intralumbar administration in the treatment of overt meningeal leukemia.[1065] However, even when methotrexate was infused continuously into the ventricle for prolonged periods in animals, steady-state drug concentrations in the lumbar space were lower than simultaneous ventricular concentrations, presumably because the rate of CSF flow into the lumbar sac is slower than the rate of diffusion or excretion of methotrexate from the CSF. [1066]

Ventricular access devices, which are reserved for patients with overt leptomeningeal disease, allow for more flexible drug administration schedules, such as the C•T schedule (daily for 3 consecutive days). C•T methotrexate and cytarabine proved to be effective for inducing and maintaining CSF remission in patients with multiply recurrent leptomeningeal leukemia. [1067]

There are a limited number of agents that are routinely administered intrathecally. The most commonly used agents are the antimetabolites methotrexate and cytarabine and the alkylating agent thiotepa. Phase I clinical trials of intrathecal delivery of the preactivated cyclophosphamide analog mafosfamide, a sustained-release formulation of cytarabine (DepoCyt), and a microcrystalline formulation of busulfan, as well as a phase II trial of intrathecal topotecan, are ongoing.

#### *Methotrexate*

Intrathecal methotrexate has been in clinical use for more than 30 years, primarily for the treatment of the meningeal spread of cancer, especially leukemia and lymphoma. It is also administered adjuvantly to patients with newly diagnosed ALL to prevent meningeal relapse. Acute and delayed neurotoxic reactions to intrathecal methotrexate have been reported. An acute chemical arachnoiditis characterized by headache, nuchal rigidity, vomiting, fever, and CSF pleocytosis can present several hours to days after a dose. A subacute encephalopathy, which may be irreversible in some patients, presents with extremity paresis and cranial nerve palsies, ataxia, visual impairment, seizures, and coma. This syndrome is associated with elevated CSF drug concentrations. [1068] An ascending radiculopathy with loss of primarily motor function resembling Guillain-Barré syndrome is also associated with intrathecal methotrexate, as well as cytarabine, and occurs days to weeks after a course of therapy.[1069] A chronic, progressive demyelinating encephalopathy (i.e., leukoencephalopathy) that appears months to years after intrathecal methotrexate leads to dementia, spastic paralysis, seizures, and coma in more advanced cases. Severe, often fatal reactions can result from the inadvertent administration of excessive doses of methotrexate or the administration of the wrong agent (e.g., vincristine) intrathecally. Great care must be taken by clinicians administering drugs by this route.[1070] Intrathecal methotrexate overdose should be treated immediately with CSF drainage or ventriculolumbar perfusion. Intrathecal instillation of carboxypeptidase-$G_2$ was also effective in animals and is now available for human use on an investigational basis. [1071]

Methotrexate elimination from the CSF after intrathecal injection is biphasic, with a terminal half-life of 14 hours. [1072] Methotrexate is eliminated by passive diffusion out of the CSF, bulk resorption of CSF, and a nonspecific active transport system. [1066] Conditions associated with delayed clearance of methotrexate from the CSF include meningeal leukemia, communicating hydrocephalus, or the lumbar puncture syndrome. [1072]

When methotrexate is administered intrathecally, the volume of the CSF is the initial volume in which the drug is distributed. In young children, CSF volume increases much more rapidly than the body surface area, reaching 80% of the adult volume by the age of 3 years. [1073] An intrathecal dose based on body surface area would underdose young children and overdose adolescents. Bleyer and colleagues recommended an intrathecal dosage schedule for methotrexate based on age instead of body surface area (Table 10-9).[1073] This regimen has been less neurotoxic, and since this dosing scheme was incorporated into front-line leukemia protocols, the CNS relapse rate has declined from 12% to 7%.[1074] The greatest decline was observed in the youngest patients, the group in whom the intrathecal methotrexate dosage was increased with the new adaptive dosing regimen.

| Age (yr) | Dose (mg) |
|---|---|
| <1 | 6 |
| 1 | 8 |
| 2 | 10 |
| ≥3 | 12 |

From Bleyer WA. Clinical pharmacology of intrathecal methotrexate. II: an improved dosage regimen derived from age-related pharmacokinetics. Cancer Treat Rep 1977;61:1419, with permission.

**TABLE 10-9. PHARMACOKINETICALLY DERIVED DOSING SCHEDULE FOR INTRATHECAL METHOTREXATE**

### *Cytarabine*

Intrathecally administered cytarabine is also of value in the treatment and prevention of meningeal leukemia. The clinical pharmacology of intrathecal cytarabine is quite different from that seen with systemic administration of this agent. With an intraventricular dose of 30 mg, peak concentrations exceed 2 mM and remain above 1 µM (a cytotoxic concentration *in vitro*) for 24 hours.[623,1075] Levels of cytidine deaminase, the enzyme that metabolizes cytarabine to ara-U, are low in brain and CSF, and metabolism to ara-U is therefore only a minor pathway of elimination.[1076] The ratio of ara-U to cytarabine in the CSF is only 0.08. The terminal half-life of cytarabine is 3.5 hours, and the clearance is 0.42 mL per minute, similar to the CSF bulk flow rate.[1075] Plasma concentrations of cytarabine after an intrathecal 30 mg dose are less than 1 µM. Leukemic cells in the CSF were found to accumulate significant levels of intracellular ara-CTP after intrathecal cytarabine, and this active metabolite was retained in cells longer (half-life of 8 to 36 hours) than in peripheral lymphoblasts.[1077] Neurotoxicity from intrathecal cytarabine includes arachnoiditis, radiculopathy, seizures, encephalopathy, or myelopathy.[1058,1078]

DepoCyt, which is a liposome-encapsulated formulation of cytarabine, was specifically developed for intrathecal use. Sustained release of cytarabine from the liposomes markedly prolongs the cytarabine half-life (141 hours) and the duration of exposure to cytotoxic concentrations (9 days) in CSF.[1079] The recommended dosage in adult patients is 50 mg, and a phase I clinical trial in children is ongoing. When given with concomitant oral dexamethasone, the toxicity profile of DepoCyt is similar to that of unencapsulated cytarabine. Acute toxicities include fever, headache, back pain, nausea, and encephalopathy.[1079,1080]

### *Thiotepa*

The alkylating agent thiotepa can be safely administered intrathecally at a dose of 10 mg and is a second-line agent for childhood meningeal cancers. Thiotepa toxicity is similar to that of intrathecal methotrexate.[1081] However, thiotepa is highly lipophilic and diffuses rapidly out of the CSF, leading to limited drug distribution within the subarachnoid space. After intraventricular administration, thiotepa is rapidly cleared from the CSF at a rate tenfold higher than CSF bulk flow, and exposure in the lumbar CSF is less than 10% of that achieved in the ventricle.[1082]

### High-Dose Systemic Therapy for Meningeal and Central Nervous System Tumors

Limited CNS penetration of some anticancer agents can be overcome by administering high doses of the drugs systemically. This approach has been successfully applied with methotrexate and cytarabine. The advantages of the systemic approach over intrathecal therapy include sustained CSF drug concentrations with prolonged intravenous infusions and better drug penetration into the deep perivascular spaces and brain parenchyma. However, methotrexate concentrations are not uniform throughout the subarachnoid space at steady state during a continuous intravenous infusion of methotrexate. The lumbar CSF concentrations are higher than ventricular CSF concentrations.[1066] The other disadvantage of this approach is the potential for severe systemic toxicity.[1056]

Very high systemic methotrexate doses can be safely delivered with leucovorin rescue, and therapeutic concentrations of methotrexate can be achieved in the CSF. A dose of 33,600 mg per $m^2$ administered over 24 hours as a loading dose (6,000 mg per $m^2$) followed by a continuous infusion (1,200 mg per $m^2$ per hour for 23 hours) results in CSF methotrexate concentrations of 30 to 40 µM.[537] The remission induction rate in patients with overt meningeal leukemia with this regimen is 80%. This regimen has also been successful as preventive therapy for meningeal leukemia in patients with ALL.[1083]

The CSF penetration of cytarabine is more favorable than methotrexate but is dose dependent. The ratio in one study decreased from 33% to 18%, with an increase in the dose from 4,000 to 18,000 mg per $m^2$ administered as a 72-hour infusion.[619] The standard high-dose (3,000 mg per $m^2$) regimen given every 12 hours results in persistent cytotoxic concentrations of cytarabine in the CSF, in part because the elimination half-life of cytarabine in CSF is eightfold longer than in plasma because of the low levels of cytidine deaminase in brain and CSF.[1084] High-dose intravenous cytarabine appears to be effective for treating CNS leukemia and lymphoma but is associated with significant systemic toxicity.[1085,1086 and 1087]

Other agents for which the systemic approach may be applicable include cyclophosphamide and thiotepa. Cyclophosphamide in high doses (80 mg per kg per day for 2 days) appears to be active against brain tumors.[1088] The systemic approach may also be more appropriate for thiotepa. After intravenous administration, plasma and CSF drug concentrations are equivalent, and significant amounts of the active metabolite, TEPA, also penetrate into the CNS.[1082]

## PERSPECTIVES

Although a high proportion of children with certain types of cancer are being cured, there are still too many who fail to respond or who relapse and eventually succumb to their cancer after a good initial response. Failure of multimodality therapy to cure individual patients may result from de novo or acquired resistance to the anticancer drugs that are used in the regimen or inadequate drug delivery to the cancer. The latter pharmacologic limitations of therapy can result from interpatient variability in drug disposition with poor absorption or more rapid drug clearance in a subgroup of patients, limiting drug exposure or dose modifications necessitated by acute and chronic toxicity.

In clinical practice, selection of chemotherapeutic combination regimens to treat individual patients is based only on tumor histology and the extent of disease. Despite the known heterogeneity in the responsiveness of tumors to single-agent therapy, a phenomenon that suggests a high incidence of de novo drug resistance, the drug sensitivity profile of individual tumors is not a determinant in the drug selection process. As a result, many patients probably receive one or more drugs that are inactive against their tumors and that produce toxicities that limit the use of other, more effective agents. Culturing tumor samples *ex vivo* and quantifying drug sensitivity, similar to the sensitivity testing routinely performed with bacterial isolates, has not proven to be practical, because sufficient tumor specimens are not readily accessible, and adequate *in vitro* growth is achieved in only a small proportion of specimens (28% of a series of pediatric tumors).[1089] Measurement of biochemical or genetic markers of drug resistance in tumor specimens is a promising potential means of quantifying drug sensitivity, but the clinical applicability of this approach must still be demonstrated.

Identifying the mechanisms of drug resistance at a molecular level may lead to the development of chemosensitizing agents, such as P-gp inhibitors. In addition, alterations that result in resistance to one agent may enhance sensitivity to the effects of a second drug that effects the same pathway.

Anticancer drug dosing is toxicity based. The optimum dose of most anticancer drugs is the maximum tolerated dose, and after this fixed dose is administered, dose modifications are based on the severity of ensuing toxicity. A more rational approach would be to individualize drug dose and schedule based on specific patient characteristics (adaptive dosing) and on plasma drug concentration (therapeutic drug monitoring). These strategies have been successfully applied to adapting carboplatin dose for renal function and to basing leucovorin rescue on methotrexate plasma concentration after high-dose methotrexate therapy. Although therapeutic and toxic drug concentrations are not known for most anticancer drugs, simply defining the average plasma concentration after a standard drug dose might help to identify outliers and produce rational dose modifications for patients with organ dysfunction.

Those patients who are cured are at risk for significant and often life-threatening acute and long-term toxic effects of the treatment. The severity of the toxicity of the anticancer drugs reflects their nonselective mechanisms of action and the emphasis on dose intensity to maximize tumor cell kill. Methods to circumvent or ameliorate chemotherapy-induced toxicity have improved the tolerability of chemotherapy. Examples of rescuing patients from dose-limiting toxicities include the use of hematopoietic growth factors, such as Filgrastim and interleukin-11, to limit the duration of granulocytopenia and thrombocytopenia after myelosuppressive therapy, the administration of mesna to block the urotoxicity of the oxazaphosphorines, leucovorin rescue from high-dose methotrexate, and the prevention of anthracycline cardiotoxicity with dexrazoxane.

The search for more selective and less toxic anticancer drugs and for more effective drug combinations must continue. Our rapidly expanding knowledge of the molecular pathogenesis of malignant transformation and tumor progression is leading to the development of more selective drugs that are designed to interfere with the critical steps in these processes. Identification and development of molecularly targeted anticancer drugs has become the primary focus of anticancer drug development.

To meet these challenges, a greater effort must be made to incorporate the advances made in the basic science of oncology and pharmacology into the design and use of chemotherapeutic treatment regimens.

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# 11

# EVOLVING MOLECULAR AND TARGETED THERAPIES

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## INTRODUCTION

The early development of agents for the treatment of oncologic diseases has focused, for most of the last 30 years, on empirical screening of new compounds, followed by dose, schedule, and toxicity profile determination. The clarification of mechanism of action as well as activity often occurs after the agent is in clinical trials and antitumor activity has been determined. None of the current drug screening panels is predictive of disease-specific tumor chemotherapy selection with the exception of chemotherapy-sensitive diseases such as leukemia and lymphoma. However, these panels are useful in identifying agents that are mechanistically similar.

The emergence of molecular-targeted therapy through rational drug design is more recent. As the molecular phenotypes of specific malignancies have been elucidated, targets for therapy have been more clearly defined. The characterization of pathways that define malignant transformation and phenotype as well as progression, invasion, and metastasis have focused new agent development on key pathways involved in angiogenesis, apoptosis, cell cycle regulation, receptor signaling, cellular proliferation, and differentiation that may be perturbed in malignant tissues compared to their normal counterparts. Alterations in tumor biologic processes mediated by oncogene activation and loss of suppressor gene function affect these pathways. Blocking oncogene function or restoring suppressor gene activity may lead to the development of tumor-specific therapy.

With the emergence of molecular-targeted therapies, preclinical assay development and validation will be critically important for agent development. Advances in molecular biology and combinatorial chemistry, using the emerging technologies of microarray, proteomics, and combinatorial libraries, have led to the identification of a variety of new agents, including small molecule inhibitors, antibodies, peptides, genes, and antisense oligonucleotides. The drug development community has used these emerging technologies to push molecular-targeted therapies ahead more rapidly. The integration of these new treatment modalities into clinical practice represents both a tremendous opportunity and a challenge for the pediatric oncologist. New clinical trial designs as well as tumor biology investigations will be required to gain an understanding of the role of these agents.

The primary objectives of this chapter are to provide information about specific classes of molecular-targeted therapies that are in clinical development and to provide an overview of issues relating to the development of targeted agents for childhood cancers. A central concept is that the ultimate benefit of molecular-targeted therapies for pediatric tumors depends on how modulation of specific targets alters the balance between tumor cell survival-signaling pathways and tumor cell death-signaling pathways in comparison to the effect of target modulation on noncancerous cells.

## CENTRAL ROLE OF THE APOPTOTIC PATHWAY

Given the goal of achieving higher levels of tumor cell kill, the application of molecular-targeted therapies for specific childhood cancers needs to be based on an understanding of the primary survival and cell death pathways for specific cancers. Extraordinary advances in cancer biology over the past decade have elucidated multiple intracellular signaling pathways that promote cancer cell survival as well as pathways that control cancer cell death. [1,2 and 3] There are two primary intracellular pathways leading to cell death (Fig. 11-1).[1] The death receptor (DR) pathway is initiated by the binding of a member of the tumor necrosis factor (TNF) receptor superfamily [e.g., CD95 (Fas), TNF receptor 1, and the TNF-related apoptosis-inducing ligand (TRAIL) receptors DR4/TRAIL-R1 and DR5/TRAIL-R2] with its ligand, leading to activation of caspase-8 and to eventual apoptosis. The mitochondrial death pathway is activated by various types of stimuli or cellular injury, leading to changes at the mitochondrial membrane causing release of cytochrome C in a multistep process. Cytochrome C binds to apoptotic protease activating factor-1 (Apaf-1) in the cytoplasm, leading to caspase-9 activation.[4] In both pathways, activation of upstream caspases, such as caspases-8 and -9, is amplified by a cascade

mechanism leading to widespread activation of downstream caspases, for example, caspase-3.

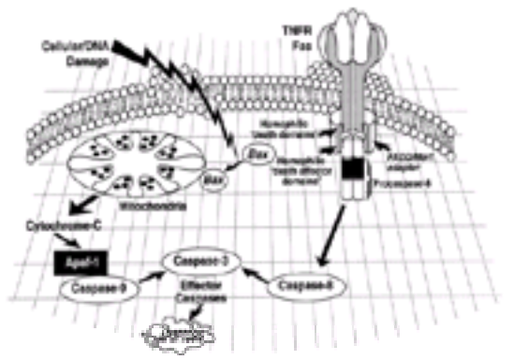



**FIGURE 11-1.** The two primary apoptotic pathways. The death receptor (DR) pathway (*right*) is initiated by ligation and clustering of members of the DR superfamily [tumor necrosis factor receptor (TNFR) I, CD95, and the TNF-related apoptosis-inducing ligand (TRAIL) receptors DR4 and DR5]. Receptor clustering leads to formation of death-inducing signal complexes by the recruitment of adapter proteins, such as Fas-activated death domain (FADD), which contain both “death domains” for interacting with the DRs and “death-affector domains” for interacting with procaspase-8. The procaspase-8 that is recruited to the complex is activated by proteolytic cleavage to active caspase-8. The mitochondrial pathway ( *left*) is responsive to internal toxic stimuli such as DNA damage. These stimuli activate pro-apoptotic members of the bcl-2 family, including Bax, Bad, Bim, and Bid, which are then attracted to the mitochondria. Their interaction with the mitochondria results in the release of cytochrome C from the mitochondria into the cytoplasm, leading to its association with apoptotic protease-activating factor 1 (Apaf-1) and subsequently procaspase-9 to form a complex, the “apoptosome,” resulting in formation of active caspase-9. In both the DR and the mitochondrial pathways, the final common pathway is activation of downstream caspases such as caspase-3.

Other pathways leading to cancer cell death are primarily extrinsic to the cancer cell. These extrinsic pathways to cell death also offer therapeutic targets. For example, cancer cells may be killed by nutrient deprivation through inhibition of angiogenesis. In pediatric tumor preclinical models, some anti-angiogenic agents have produced tumor regression, with particularly impressive activity when these agents are combined with agents targeting the tumor cell population. [5,6]

Counterbalancing the signaling pathways leading to cell death are multiple factors and signaling pathways that favor cell survival ( Fig. 11-2), including: (a) anti-apoptotic members of the bcl-2 family that inhibit mitochondrial release of cytochrome C and other apoptogenic factors [7]; (b) anti-apoptotic members of the immunosuppressive acidic protein family that inhibit activated caspases; (c) survival signals that are initiated at the cell membrane by activation of growth factor receptors and that are mediated intracellularly by phosphoinositide-3-kinase (PI3'K) activation, resulting in activation of the serine/threonine kinase Akt [8]; and (d) activation of nuclear factor-kB (NF-kB).[9,10] and [11]

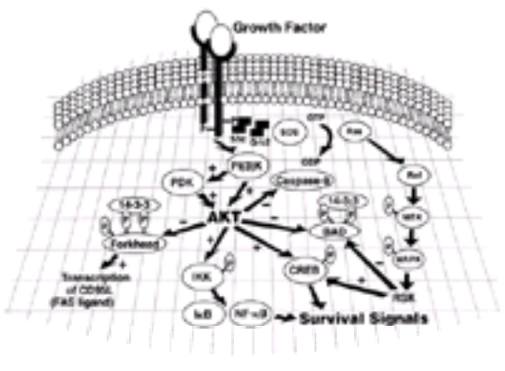



**FIGURE 11-2.** Survival signaling pathways. Growth factors and other stimuli produce signals that promote cell survival over apoptosis. The phosphoinositide 3-kinase (PI3'K) pathway plays a central role in survival signaling through activation of the Akt serine-threonine kinase. Activated Akt phosphorylates a number of proteins involved in either survival or apoptotic pathways: the pro-apoptotic protein Bad, leading to its inactivation and cytoplasmic sequestration by association with 14-3-3 proteins; caspase-9, leading to its inactivation and to suppression of caspase-9–induced cell death; members of the forkhead family of transcription factors, leading to their interaction with 14-3-3 proteins and their cytoplasmic sequestration, resulting in reduced transcription of death genes such as Fas ligand; IKK, leading to enhanced degradation of the IkBs and to nuclear factor-kB (NF-kB) activation and transcription of survival-promoting genes; the cAMP response element binding protein (CREB) transcription factor, leading to increased transcription of bcl-2. Survival is also promoted through the mitogen-activated protein kinase (MAPK) pathway, which is activated by growth factors in a pathway involving Ras. Survival signals through the MAPK pathway are provided in part through activation of pp90 ribosomal S6 kinase (Rsk) family members. Similar to Akt, Rsk family members can phosphorylate and activate CREB and can phosphorylate Bad, leading to its inactivation. GDP, guanosine diphosphate; GTP, guanosine triphosphate; P, phosphate.

## G3139: Bcl-2-Targeted Therapy

### *Background*

Bcl-2 inhibits apoptosis and is an important contributor to chemoresistance for some cancers. For these cancers, blocking the anti-apoptotic activity of bcl-2 may increase the efficacy of cytotoxic therapy. G3139 is an 18mer phosphorothioate oligonucleotide that was designed to bind to the first six codons of the human bcl-2 messenger RNA (mRNA). G3139 down-regulates bcl-2 mRNA leading to reduced bcl-2 protein levels *in vitro*.[12] Bcl-2 antisense oligonucleotides have been shown to sensitize acute myelogenous leukemia (AML) cells and lymphoma cells to conventional cytotoxic agents such as cytarabine (Ara-C) and methotrexate. [13,14] In tumor xenograft models, G3139 was active as a single agent against follicular non-Hodgkin's lymphoma (NHL), [12] Merkel cell carcinoma,[15] and Epstein-Barr virus–associated lymphoproliferative disease.[16] G3139 enhanced the therapeutic efficacy of cyclophosphamide in a severe combined immunodeficiency disease mouse model of human NHL[17] and also chemosensitized human melanoma in severe combined immunodeficiency disease (SCID) mice.[18]

### *Clinical Experience*

Initial clinical trials of G3139 in adults used outpatient subcutaneous 21-day infusions. [19] The maximum tolerated dose (MTD) was 147.2 mg/m$^2$/day (approximately 5 mg/kg/day), with dose-limiting toxicities of thrombocytopenia, hypotension, fever, and asthenia. Plasma levels of G3139 equivalent to the efficacious plasma concentration (1.0 µg/mL) determined in *in vivo* models were achieved. Bcl-2 protein expression was reduced in tumor cells derived from lymph nodes in two patients and peripheral blood or bone marrow mononuclear cell populations from five patients.

A rational use of G3139 based on its mechanism of action is to combine it with chemotherapy to overcome bcl-2–associated resistance to chemotherapy. G3139 has been studied in phase I trials with several chemotherapy agents, including mitoxantrone, [20] docetaxel,[21] and paclitaxel.[22] G3139 has also been combined with fludarabine and high-dose Ara-C for the treatment of refractory/recurrent AML, [23] with G3139 given by continuous infusion on days 1 to 10 and with fludarabine and Ara-C given on days 6 to 10. Complete responses were observed in three of the first ten patients, and toxicities were not substantially different from those anticipated for a high-dose Ara-C regimen.

### *Pediatric Applications*

Pediatric tumors for which bcl-2–directed therapy may be warranted include neuroblastoma and AML. Immunopositivity for bcl-2 expression has been demonstrated for primary and metastatic sites of neuroblastoma tumors and in pre- and postchemotherapy tumor samples.[24] An association of higher bcl-2 expression with unfavorable histology and N-*myc* gene amplification has been reported in some studies, suggesting an association with poor prognosis.[25,26 and 27] For AML, bcl-2 overexpression has been found in 61% of adults at diagnosis and 74% of patients at relapse.[28] High expression of bcl-2 was associated with a poorer response to chemotherapy and shorter survival.[29,30,31 and 32] A complicating factor in assessing the role of bcl-2 in resistance to chemotherapy is that after chemotherapy, bcl-2 expression levels in tumor cells may increase, possibly as a result of preferential killing of cells with low bcl-2 expression.[33] For patients with AML, high levels of bcl-2 have been demonstrated in residual leukemia cells from patients in clinical remission.[33] These findings suggest that correlations between bcl-2 measurements and clinical outcomes may be limited by the ability to accurately measure bcl-2 at diagnosis in subpopulations of cancer cells that could lead to relapse.

### Rituximab: A Signaling Monoclonal Antibody That Induces Apoptosis

#### *Background*

Rituximab is a highly specific mouse/human chimeric antibody engineered by grafting the variable regions targeting the CD20 antigen from murine antibody genes onto the constant regions of human immunoglobulin (Ig) G genes. The CD20 antigen is a 35,000-d phosphoprotein present exclusively on B cells (pre-B and mature B lymphocytes) and present on most B-cell lymphomas.[34,35] Rituximab can kill cells by triggering antibody-dependent cellular toxicity, by activating the complement cascade,[36] and by triggering intracellular signaling pathways leading to apoptosis.[37,38] The latter activity may contribute to rituximab's ability to enhance the activity of chemotherapy agents.[39,40]

#### *Clinical Experience*

The initial trials of rituximab targeted adults with recurrent low-grade lymphomas and used a weekly schedule of administration. High levels of activity were observed, particularly for patients with follicular center cell lymphoma.[41] Rituximab was also shown to be active against diffuse large B-cell lymphoma (DLCL).[42] Rituximab has been safely combined with standard CHOP [cyclophosphamide, hydroxydaunomycin, vincristine (Oncovin), prednisone] chemotherapy for patients with low-grade or follicular B-cell NHL.[43] A very high overall response rate was observed (55% complete remission and 40% partial remission), with seven of eight bcl-2–positive patients converting to PCR negativity in blood and marrow (molecular complete remissions).[43] Patients with previously untreated intermediate- and high-grade NHL were treated with rituximab given on day 1 and CHOP chemotherapy given on day 3.[44] A promising overall response rate of 97% was observed, with no appreciable increase in toxicity compared to CHOP alone. Building on these promising results, a randomized trial in elderly adults with DLCL comparing CHOP to CHOP plus rituximab demonstrated significantly higher event-free survival and survival rates for patients receiving rituximab plus chemotherapy.[45]

Toxicities attributed to rituximab include infusional or allergic reactions, B-cell depletion, neutropenia, and thrombocytopenia. Tumor lysis syndrome has also occurred in patients with large tumor burden or high circulating lymphocyte counts.[46,47 and 48] Most of these side effects are mild or manageable, but rarely, life-threatening or fatal infusional reactions, tumor lysis syndromes, and skin reactions have been reported.

#### *Pediatric Applications*

The primary cancer diagnoses for which rituximab may be relevant are the B-immunophenotype NHLs, particularly diffuse large B-cell NHL (DLCL) and Burkitt's lymphoma. Both Burkitt's lymphoma cells and DLCL cells express high levels of CD20.[49,50,51,52,53 and 54] Rituximab induces apoptosis in Burkitt's cell lines,[55] apparently via signal transduction pathways that involve influx of $Ca^{2+}$, Src family kinase activation, phospholipase C gamma 2 (PLCg2) phosphorylation, activation of the mitogen-activated protein kinase (MAPK) family members p44 (ERK1) and p42 (ERK2), up-regulation of the pro-apoptotic protein Bax, and activation of caspase-3.[37,38] Anti-CD20 monoclonal antibodies are active against Burkitt's lymphoma in a xenograft nude mouse model.[56]

Pediatric clinical experience with rituximab in children with Burkitt's lymphoma is restricted to anecdotal reports of responses in patients who progressed after intensive chemotherapy.[57] Rituximab has been used to treat children with posttransplant lymphoproliferative disease with a high rate of complete responses.[58,59 and 60] This experience suggests that rituximab can be given safely to children at the standard doses used for adult patients and that unanticipated toxicities have not yet been observed in children. Evaluation of rituximab in combination with standard chemotherapy agents is planned for children with Burkitt's lymphoma and DLCL.

## GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITORS

Receptor tyrosine kinases (RTKs) include the cell surface receptors for growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor-a (TGF-a), and insulin-like growth factor-1 (IGF-1).[61] The RTKs are glycoproteins with an extracellular ligand-binding domain, one membrane-spanning domain and a conserved cytoplasmic domain containing a site with tyrosine kinase activity ( Fig. 11-3). Binding of ligand to RTKs leads to receptor dimerization, which triggers phosphorylation or auto-phosphorylation of tyrosine residues in the cytoplasmic domains ( Fig. 11-3). These phosphorylated residues become attachment sites for a variety of adaptor molecules, leading to activation of signal transduction pathways that modulate cell growth, differentiation, and survival. Relatively specific inhibitors of these pathways have entered clinical evaluation, including small molecule inhibitors and monoclonal antibodies directed against the cell surface receptors (Table 11-1 and Table 11-2 list agents under evaluation). An example from each class is discussed in the following sections.

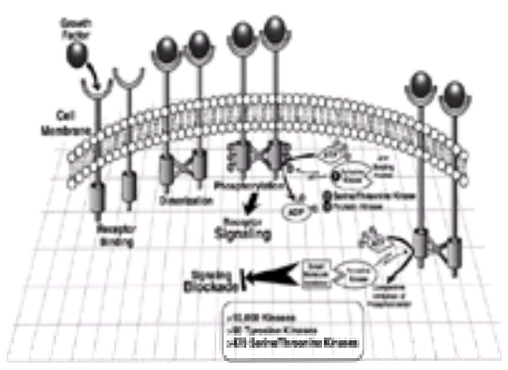
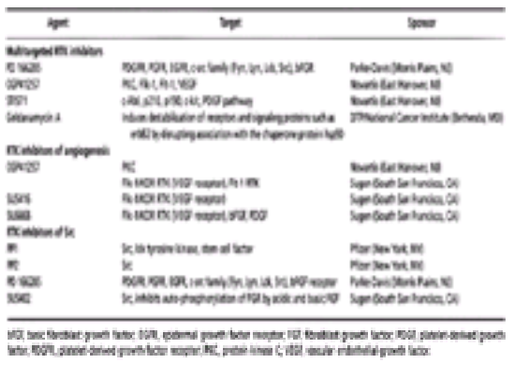



**FIGURE 11-3.** The protein, tyrosine, and serine/threonine kinases are effectors of signal transduction activated by phosphorylation. Growth factors bind to the extracellular portion of the receptor. Subsequent to receptor binding, dimerization occurs. The receptor autophosphorylates the conserved intracellular receptor domain. The RTK intracellular domain is enlarged to demonstrate its intrinsic kinase activity and ATP binding pocket. After autophosphorylation, signal transduction occurs. Most small molecule kinase inhibitors compete for binding in the ATP-binding pocket of the receptor. Inhibition may be reversible or irreversible. Signal transduction may be blocked by receptor tyrosine kinase inhibitors. ADP, adenosine diphosphate; P, phosphate.

| | |
|---|---|
| **EGFR family RTK antibody inhibitors** | |
| Trastuzumab/Herceptin | Genentech (San Francisco, CA) |
| C225 | Imclone Systems (New York, NY) |
| ABX-EGF | Abgenix (Fremont, CA) |
| E7.6.3 | Pfizer (New York, NY) |
| EMD 55 900 | Pfizer (New York, NY) |
| ICR62 | — |
| TheraCIM h-R3 | York Medical |
| **EGFR reversible small molecule inhibitors** | |
| ZD1839 | AstraZeneca (Wilmington, DE) |
| OSI 774/CP 358 774 | OSI Pharmaceuticals (Birmingham, UK) |
| CGP 75166/PKI166 | Novartis (East Hanover, NJ) |
| CGP 59326A | Novartis (East Hanover, NJ) |
| BIBX 1382 | Boehringer Ingelheim (Ridgefield, CT) |
| **EGFR irreversible small molecule inhibitors** | |
| CI-1033/PD183805 | Parke-Davis (Morris Plains, NJ) |
| EKB-569 | Wyeth-Ayerst (Philadelphia, PA) |

**TABLE 11-1. RECEPTOR TYROSINE KINASE (RTK) INHIBITORS: EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) INHIBITORS IN CLINICAL DEVELOPMENT**



| Agent | Target | Sponsor |
|---|---|---|
| **Multitargeted RTK inhibitors** | | |
| PD 166285 | PDGFR, FGFR, EGFR, c-src family (Fyr, Lyn, Lck, Src), bFGF | Parke-Davis (Morris Plains, NJ) |
| CGP41251 | PKC, Raf-1, Flt-1, VEGF | Novartis (East Hanover, NJ) |
| SU5271 | c-Met, p21ras, p70S, c-Akt, PDGF pathway | Novartis (East Hanover, NJ) |
| Geldanamycin A | Induces destabilization of receptor and signaling proteins such as erbB2 by disrupting association with the chaperone protein hsp90 | DTP/National Cancer Institute (Bethesda, MD) |
| **RTK inhibitors of angiogenesis** | | |
| CGP41251 | PKC | Novartis (East Hanover, NJ) |
| | Flk KDCR RTK (VEGF receptor), Flt-1 RTK | Sugen (South San Francisco, CA) |
| SU5416 | Flk KDCR RTK (VEGF receptor) | Sugen (South San Francisco, CA) |
| SU6668 | Flk KDCR RTK (VEGF receptor), bFGF, PDGF | Sugen (South San Francisco, CA) |
| **RTK inhibitors of Src** | | |
| CP-1 | Src, kit tyrosine kinase, stem cell factor | Pfizer (New York, NY) |
| CP-2 | Src | Pfizer (New York, NY) |
| PD 166285 | PDGFR, FGFR, EGFR, c-src family (Fyr, Lyn, Lck, Src), bFGF receptor | Parke-Davis (Morris Plains, NJ) |
| SU6482 | Src, inhibits auto-phosphorylation of PDGF by acidic and basic FGF | Sugen (South San Francisco, CA) |

bFGF, basic fibroblast growth factor; EGFR, epidermal growth factor receptor; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PKC, protein kinase C; VEGF, vascular endothelial growth factor.

**TABLE 11-2. RECEPTOR TYROSINE KINASE (RTK) INHIBITORS: MULTITARGETED INHIBITORS IN PRECLINICAL OR CLINICAL DEVELOPMENT**

**Trastuzumab: ErbB-2 Signaling Inhibitor**

***Background***

Trastuzumab (Herceptin) is a humanized monoclonal antibody that selectively binds with high affinity to the extracellular domain of ErbB-2. ErbB-2 (Her2 or *neu*) is a member of the EGF receptor (EGFR) family of type-1 RTKs. Although a high-affinity ligand for ErbB-2 has not been identified, [62] ErbB-2 can serve as a heterodimerization partner for other members of the EGFR family including EGFR, ErbB-3, and ErbB-4. [63] By serving as an essential component of these receptor complexes, ErbB-2 plays a central role in controlling cell growth, differentiation, and apoptosis. [63,64]

ErbB-2 is overexpressed in many adult cancers, including breast, ovarian, gastric, lung, and prostate cancers. ErbB-2 provides a survival signal in cancer cells in which it is overexpressed.[65] It appears to mediate resistance to the cytotoxic effects of TNF,[66] chemotherapy,[67] radiation therapy,[68,69] and hormonal therapy.[70] Overexpression of ErbB-2 in women with breast cancer, which is usually a result of amplification of the ErbB-2 gene on chromosome 17, is associated with poor outcome.[71]

Trastuzumab appears to act primarily as an anti-proliferative agent when used to treat ErbB-2 overexpressing cancer cells *in vitro* or *in vivo*.[64] Trastuzumab-mediated growth inhibition may be due to interference with the interaction of ErbB-2 with other EGFR family members [72] or may be caused by acceleration of ErbB-2 degradation.[73] Trastuzumab is also capable of activating complement and can trigger antibody-dependent cell-mediated cytotoxicity. [64] Trastuzumab enhances the antitumor activity of a variety of chemotherapy agents both *in vitro* and *in vivo*.[74,75 and 76]

***Clinical Experience***

Trastuzumab has been tested primarily in patients with breast cancer. The agent has generally been given on a weekly schedule. [77] Trastuzumab induced objective responses in approximately 15% of women with metastatic breast cancer whose tumors overexpressed ErbB-2.[77] In a randomized phase III study of cyclophosphamide and doxorubicin or paclitaxel administered in the presence or absence of trastuzumab, the addition of trastuzumab to chemotherapy increased response rate, time to progression, and survival in breast cancer. [78,79]

The primary toxicity associated with trastuzumab as a single agent was infusion-associated fever or chills that usually occurred only during the first infusion. [77] When given with chemotherapy, trastuzumab was generally well tolerated except for cardiac dysfunction, which was more common for patients receiving concurrent doxorubicin (19%) than for patients receiving concurrent paclitaxel (4%). [79] This exacerbation of chemotherapy-induced cardiac toxicity by trastuzumab is likely related to its interference with the role of ErbB-2 in mediating the activation of important cardiac survival pathways. [80] ErbB-2 and other members of the EGFR family are expressed on cardiac cells, both during development and postnatally. [81] They play essential roles in heart development [82,83] and cardiac myocyte survival and protection from apoptosis.[81]

***Pediatric Applications***

The primary pediatric tumors for which ErbB-2 may play a substantial role in tumor cell growth and survival are osteosarcoma [84,85] and medulloblastoma.[86,87] Because of the limited penetration of monoclonal antibodies into the cerebrospinal fluid, evaluation of systemically administered trastuzumab for medulloblastoma is not planned. ErbB-2 is expressed on approximately 40% of osteosarcoma tumor specimens,[84,85] with expression being more frequent in tumors of patients with metastatic disease at presentation.[84] For patients with nonmetastatic disease, ErbB-2 expression was associated with higher risk of treatment failure. [84] Pediatric development plans for trastuzumab include a phase II study in patients with recurrent osteosarcoma and a pilot study in combination with chemotherapy for patients with newly diagnosed metastatic osteosarcoma.

**ZD1839: Epidermal Growth Factor Receptor Small Molecule Inhibitor**

***Background***

EGFR (ErbB-1) and its ligands, EGF and TGF-a, are important in tumor cell proliferation and survival, and are also involved in motility, adhesion, invasion, and angiogenesis.[88] EGFR is overexpressed in a variety of epithelial malignancies, including glioma, non–small cell lung cancer (NSCLC), breast, head and neck, bladder, and ovarian carcinomas.[89] Overexpression is often associated with poorer prognosis.[88,89]

Inhibition of EGFR signaling can lead to cell cycle arrest and to apoptosis in cells that express EGFR. [88,90] Growth inhibition by EGFR inhibitors is associated with up-regulation of the cyclin-dependent kinase (cdk) inhibitor p27. [90,91] Inhibitors of EGFR can induce growth delay and, less commonly, regressions in tumor xenograft models.[92,93] EGFR expression may not be as important for antitumor effect as the tumor cell's reliance on this pathway for cellular proliferation and survival. [61,92,94] EGFR inhibitors potentiate the cytotoxic activity of chemotherapy [92,93,95] and radiation therapy,[96,97] a characteristic with important clinical ramifications.

The EGFR inhibitors with the most clinical data are the small molecule inhibitor ZD1839 [98] and the monoclonal antibody C225,[99] an IgG antibody that binds to the ligand-binding domain of the EGFR and competes for receptor binding. Other EGFR inhibitors under development are listed in Table 11-1.

***Clinical Experience***

ZD 1839 is a potent and specific inhibitor of EGFR tyrosine kinase activity that inhibits EGFR auto-phosphorylation and signaling at submicromolar concentrations. [99] Based on the observation from preclinical xenograft models that tumor regrowth occurs rapidly when ZD1839 is discontinued, chronic administration schedules are being evaluated. ZD1839 was evaluated in the phase I setting using both an intermittent schedule (14 days on, 14 days off) [100] and a continuous dosing schedule.[101] ZD1839 was generally well tolerated with few grade 3 to 4 adverse events observed. The most common grade 1 to 2 adverse events were diarrhea and a

characteristic acne-like skin rash. Grade 3 diarrhea developed at the 700 mg/day dose after 1 week of treatment in two of nine patients treated on the intermittent schedule, but it rapidly resolved with drug cessation. [100] The mean maximal drug concentration ($C_{max}$) after 14 days of therapy ranged from 113 to 2,255 ng/mL (0.5 to 5.0 µM) for patients receiving 50 to 700 mg/day and was proportional across the entire dose range. [100] The mean elimination half-life was 34 to 46 hours, supporting a once-daily administration schedule. Steady state levels were reached by day 7. At the dose of 400 mg/day, all patients had trough plasma concentrations greater than the *in vitro* 90% inhibitory concentration in an EGF-stimulated KB human oral squamous carcinoma growth assay. Objective responses were observed in patients with NSCLC at doses as low as 150 mg/day.[100,101] Phase III trials of ZD1839 as a single agent in adults are using the continuous daily schedule, and regimens combining ZD1839 with chemotherapy and with radiation therapy are being developed. [98]

### *Pediatric Applications*

Potential target tumors for agents such as ZD1839 that inhibit EGFR signaling include neuroblastoma, [102] rhabdomyosarcoma,[102] and high-grade gliomas.[103] Inhibition of EGFR signaling by ZD1839 or by antisense oligonucleotides repressed the growth of rhabdomyosarcoma cell lines. [102,104] Pediatric high-grade gliomas rarely show the EGFR gene amplification observed in adult gliomas,[103,105] but overexpression of the receptor was observed in 80% of pediatric high-grade gliomas. [103] The eventual utility of agents such as ZD1839 for pediatric tumors expressing EGFR will likely be in combination with chemotherapy and radiation therapy, because agents that inhibit EGFR signaling can potentiate the cytotoxic activity of these modalities. [93,106,107]

## STI571: Targeting the Bcr-Abl Fusion Protein and Other Tyrosine Kinases

### *Background*

STI571 is a small molecule tyrosine kinase inhibitor developed to target the tyrosine kinase activity of the p210 $^{Bcr\text{-}Abl}$ and p190$^{Bcr\text{-}Abl}$ fusion proteins associated with chronic myeloid leukemia (CML) and Philadelphia chromosome–positive ($Ph^+$) acute lymphoblastic leukemia (ALL).[108,109] The fusion proteins esult in constitutive tyrosine kinase activity, which appears to be a crucial event in neoplastic transformation. [110,111] STI571 also inhibits the tyrosine kinase activity of the PDGF receptor and c-kit at submicromolar concentrations. [112] STI571 inhibits the growth of cells expressing the Bcr-Abl fusion protein [108] and induces apoptosis of Bcr-Abl–positive cells,[108] showing activity both *in vitro*[108,113,114] and *in vivo*.[115]

Of particular relevance to the pediatric setting and the treatment of $Ph^+$ ALL in children, STI571 selectively inhibits proliferation and Bcr-Abl phosphorylation in ALL cells expressing the p190$^{Bcr\text{-}Abl}$ protein.[109] Bcr-Abl expression exerts protection against apoptosis induced by cytotoxic agents, [116,117 and 118] and this Bcr-Abl–driven chemoresistance may contribute to the poor outcome of patients with $Ph^+$ leukemia treated with conventional chemotherapy agents. STI571 potentiates the activity of cytotoxic agents against Bcr-Abl–expressing cells, [119,120] thereby providing the strategy for combining STI571 with chemotherapy agents for Bcr-Abl–expressing leukemias. STI571 also shows antitumor activity in preclinical models of tumors in which the PDGF receptor plays a central role, including high-grade gliomas [121] and dermatofibrosarcoma protuberans.[122]

### *Clinical Experience*

In the initial phase I trial of STI571 the agent was administered orally once per day and included patients with interferon-refractory chronic phase CML. [123] STI571 was rapidly absorbed, with a terminal half-life of 10 to 23 hours and with an increase in mean plasma area under the curve (AUC) values that were proportional to the administered dose up to a dose of 750 mg.[124] The $C_{max}$ and trough levels exceeded levels associated with activity in preclinical models. No dose-limiting toxicity (DLT) has been reported with the agent. Myelosuppression, neutropenia, and thrombocytopenia occurred occasionally at doses greater than or equal to 300 mg. [124,125] For patients with chronic phase CML, complete hematologic responses occurred in 100% and complete and major cytogenetic responses occurred in 35% of those treated with doses of STI571 of 300 mg or greater.[124,125] STI571 has been less effective in CML blast crisis and in $Ph^+$ ALL, with lower remission rates and shorter response durations observed.[124,126] Ongoing evaluations of STI571 in combination with cytotoxic agents will determine whether the synergistic increase in activity for STI571 with chemotherapy agents observed in preclinical models can be translated into the clinical setting.

### *Pediatric Applications*

STI571 warrants evaluation in children with $Ph^+$ (Bcr-abl–expressing) leukemias for multiple reasons, including its unique mechanism of action, its high level of activity with minimal associated toxicity in adults with CML, and its synergistic activity in combination with chemotherapy agents. Pediatric development plans for STI571 for these leukemias include single-agent evaluation for patients with chronic phase CML and evaluations in combination with standard chemotherapy agents for patients with $Ph^+$ ALL or with CML in blast crisis.

STI571 may also have a role for pediatric tumors in which PDGF signaling may play a role in tumor cell survival and growth. Tumors for which PDGF signaling may be important include high-grade gliomas, [121,127] neuroblastoma,[128,129] desmoplastic small round cell tumors,[130,131] alveolar rhabdomyosarcoma,[132] and osteosarcoma.[133,134] Pediatric tumors that have c-kit/stem cell factor–signaling pathways are a third group of cancers that may be targets for STI571 evaluation. A substantial percentage of pediatric AML cases express c-kit.[135] Ewing's sarcoma and peripheral neuroectodermal tumor cell lines express c-kit and stem cell factor, and c-kit expression protects tumor cells against apoptosis.[136] Rhabdomyosarcoma cell lines express c-kit and stem cell factor, but blocking stem cell factor signaling did not alter growth of the cell lines.[137] Neuroblastoma cells also expressed c-kit, [138] and stem cell factor appears to provide a survival signal for these cells. Pediatric studies with STI571 are planned for children with recurrent Ewing/PNET, neuroblastoma, osteogenic sarcoma, and desmoplastic round cell tumors as well as a study of children with high-grade glioma.[139]

## FARNESYL TRANSFERASE INHIBITORS AND THE FUNCTIONAL INHIBITION OF RAS SIGNALING

The role of Ras activation in many cancers has been an impetus to develop therapeutic strategies for Ras inhibition. [140,141] Activating Ras mutations occur in approximately 30% of human cancers, being especially prevalent in pancreatic adenocarcinomas and also occurring commonly in colorectal carcinomas, thyroid carcinoma, and acute myeloid leukemia.[142] Ras may also be persistently activated by upstream growth factors or oncogenic proteins. Ras function can be inhibited by blocking mitogenic signal transduction, by affecting Ras protein farnesylation, or by inhibiting the downstream regulatory factor molecules or effectors of Ras ( Fig. 11-4).

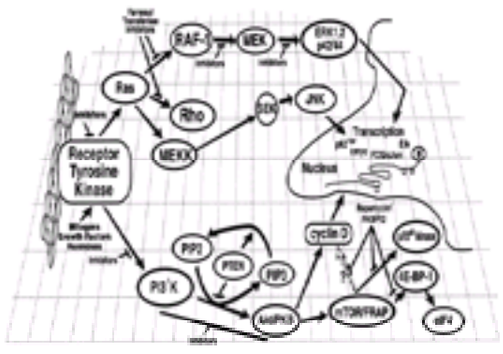



**FIGURE 11-4.** Sites at which molecularly targeted new agents inhibit the Ras and PI3'K signal transduction pathways to modulate transcription and translation are indicated by blunt-ended arrows. Blockade of receptor signaling at multiple steps in these pathways diminishes or blocks transcription activation, disrupting cellular genetic activity.

Ras, like many proteins, is farnesylated by the same farnesyl transferase (FTase) as other cellular proteins. FTase inhibitors (FTIs) are not exclusively Ras-specific. The development of FTIs was initially driven by the premise that their primary mechanism of action was inhibition of Ras isoprenylation, thereby inhibiting Ras signaling. More recent evidence suggests that the activity of FTIs may be related to alterations in RhoB, an endosomal protein that functions in receptor trafficking. [143] Treatment of cancer cells with FTIs leads to diminished levels of farnesylated RhoB and increased levels of geranylated RhoB. [144] The latter is able to inhibit survival signaling pathways in some cell types[145] and in some fibroblast models appear necessary for the apoptotic and antineoplastic responses to FTIs. [146] Activation of the PI3'K-Akt pathway may block the apoptotic effects of FTIs.[147] These observations complicate efforts to identify molecular characteristics of tumors cells that are predictive for response to FTIs.

Agents that inhibit the farnesylation reaction of FTase may be divided into three broad categories: the farnesyl diphosphate (FDP) analogs that compete with the substrate FDP for FTase, the peptidomimetics that compete with the CAAX portion of Ras for FTase, and the bi-substrate inhibitors that combine the properties of FDP analogs and peptidomimetics in a single molecule (Table 11-3).[140,142] Agents in each of these classes show *in vitro* activity against a range of tumor cell lines, including cell lines with wild-type Ras.[142] Tumor cells with mutant H-Ras appear to be more sensitive than cells with mutant K-Ras or N-Ras, perhaps reflecting differences in protein synthesis among the Ras isoforms, affecting the ability of the latter to more readily undergo geranylation when farnesylation is blocked. [148] *In vivo* antitumor activity (including regressions for some tumors) has been observed for different classes of FTIs, including the non-peptide CAAX peptidomimetics SCH66336,[149] R115777,[150] BMS-214662,[151,152] and the CAAX peptidomimetic L-744,832.[153,154]

| Agent | Target |
|---|---|
| Bi-substrate inhibitors | Combine the features of both the FDP analog and the peptidomimetics |
| FDP analogs | Compete with the substrate FDP for FTase |
| Inhibitors of farnesyl protein transferase | |
| R115777 | Tricyclic FTase |
| SCH66336 | FTase inhibitor, pyridoben zocycloheptine |
| Peptidomimetics (CAAX Mimetics) | |
| Downstream of RAS | |
| ISIS 5732/CGP69846A | c-raf kinase antisense oligonucleotide |
| PD 184352 | MEK |
| 98059 | MEK |
| U-0126 | MEK |
| ISIS 3521 | PKC, oligonucleotide |
| Bryostatin-1 | PKC |
| Other | |
| ISIS-2503 | H-Ras-antisense oligonucleotide |

FDP, farnesyl diphosphate; PKC, protein kinase C.

**TABLE 11-3. PROTEIN FARNESYL TRANSFERASE (FTase) INHIBITORS IN PRECLINICAL AND CLINICAL DEVELOPMENT**

### R115777 and SCH66336: Farnesyl Transferase Inhibitors

#### *Background*

A challenge in studying the FTIs in the clinic is defining the relationship between agent dose, serum levels, and agent effect on the target enzyme. This has been accomplished by direct measurements of FTase activity[155] and by developing assays for unprocessed forms of farnesylated proteins. [156] Drug-induced increases in the unfarnesylated species of the chaperone protein HDJ-2 and the intranuclear intermediate filament protein lamin A can be detected after treatment *in vitro* with FTIs.[156] Immunohistochemical detection of prelamin A in buccal mucosa cells of patients treated with FTIs has been used to confirm inhibition of protein farnesylation *in vivo*.[157] Because only two FTIs, R115777 and SCH66336, are currently under clinical evaluation in children, the description of clinical experience with FTIs is restricted to these two agents.

#### *Clinical Experience*

R115777 is an orally administered FTI that is competitive for the CAAX peptide substrate. [147] The agent has been studied in phase I trials on several different schedules, including twice daily × 5 days repeated every 14 days, [158] twice daily × 21 days repeated every 28 days,[159,160] and chronic twice-daily dosing.[161] These studies have demonstrated acceptable gastrointestinal absorption, an approximately 5-hour plasma half-life, and biologically relevant steady state plasma levels as predicted preclinically.[158,159] The primary toxicities for the 5-day treatment regimen were nausea, vomiting, headache, fatigue, anemia, hypotension, and reversible nephrotoxicity; only one case of DLT (peripheral neuropathy) was observed at doses up to 1,300 mg (approximately 750 mg/m $^2$).[158] By contrast, more prolonged dosing produced dose-limiting myelosuppression at a dose of 300 mg/m $^2$.[159] Complete responses were observed in the adult phase I leukemia study of R115777.[160]

SCH66336 is a potent inhibitor of farnesylation. Phase I studies in adults have used a twice-daily [157,162] or daily[163] dosing schedule. Administration of SCH66336 twice daily for 7 days resulted in dose-limiting gastrointestinal toxicity (nausea, vomiting, and diarrhea) and fatigue at a dose of 400 mg, and a dose of 350 mg was considered the recommended phase II dose.[157] Continuous daily oral administration of SCH66336 was studied in patients with miscellaneous solid tumors. [162] Doses of 300 mg or greater were not tolerated because of myelosuppression and neurotoxicity (confusion and disorientation). The recommended phase II dose was 200 mg twice daily. This dose caused mild toxicities, including diarrhea, anorexia, fatigue, and asymptomatic bradycardia. Similar results were obtained using a 14-day treatment schedule followed by 14 days of rest.[164] Using the presence of prelamin A in buccal mucosa cells as a marker for physiologically relevant FTase inhibition, *in vivo* inhibition of protein farnesylation was demonstrated at doses from 200 mg to 400 mg.[157] A partial response was observed in a patient with NSCLC.[157]

#### *Pediatric Applications*

As noted above, the activity of the FTIs may not be tightly associated with their effect on Ras isoprenylation and on Ras activation. However, FTIs have been considered for evaluation historically in pediatric tumors that have aberrant activation of the Ras pathway, either through changes in intracellular components of the Ras pathway or through inappropriate Ras activation initiated by growth factor receptors. For juvenile myelomonocytic leukemia (JMML), aberrant regulation of the Ras pathway occurs in some cases, either by Ras-activating mutations[165] or by inactivating mutations of neurofibromin,[166] a GTPase-activating protein that inhibits Ras activity. JMML cells show greater sensitivity to FTI-mediated growth inhibition than do normal myeloid precursor cells. [167] A phase II evaluation of R115777 for children with JMML is planned. Evaluations are also planned for R115777 in childhood acute leukemias, based in part on the activity observed for R115777 in adults with acute leukemia.[160]

FTIs have shown significant anti-proliferative effects in human malignant glioma cells *in vitro* at low micromolar concentrations.[168] *In vivo* antitumor activity for FTIs against human glioma cells has been demonstrated in xenograft models.[169] Of additional significance to the treatment of brain tumors, oncogenic Ras appears to contribute to radiation resistance in human tumors, and inhibition of oncogenic Ras activity by FTIs enhances the radiation sensitivity of these cells. [170,171] Given these observations, a pediatric evaluation of SCH66336 is planned for children with brain tumors.

FTIs are also of interest for patients with neurofibromatosis 1 because mutations in neurofibromin lead to increased Ras signaling. [172,173] Potential applications of FTIs for this patient population include treatment of plexiform neurofibromas[174] and treatment of neurofibromatosis 1–associated malignancies.[175]

## CELL CYCLE: CHECKPOINT CONTROL, REGULATION, AND MODULATION

### Cell Cycle

The first gap phase of the cell cycle ($G_1$) is defined by the commitment of the cell to enter the cell cycle and molecular preparation for the rigorous duplication of genomic DNA encoded on the chromosomes (Fig. 11-5). Mitogenic stimulation initiates $G_1$ progression, which can in turn be inhibited by cytokine blockade.[176,177] and [178] $G_1$ is followed by the synthetic phase of the cell cycle (S) in which the DNA (the genome) is duplicated. S phase is followed by the second gap phase of the cycle ($G_2$) during which the cell prepares to enter the mitotic phase (M), also known as *cell division*. M phase is the time during which the duplicated chromatids segregate into

the daughter cells. Mitosis may be followed by $G_0$, a phase in which DNA replication is quiescent and the cell is not committed to cycle. [179]

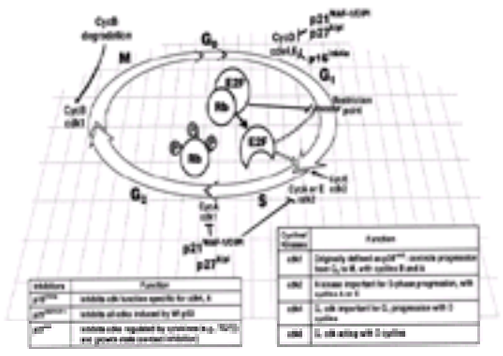


**FIGURE 11-5.** Cell cycle regulation by cyclin-dependent kinases and cyclins. The modulation of cell cycle transition is tightly controlled by E2F release from Rb and Rb phosphorylation. The p53 effector proteins p21 $^{WAF-1/CIP1}$, p27$^{Kip1}$, and p16$^{INK4a}$ inhibit the cyclins and cyclin-dependent kinases critical for cell cycle phase and restriction point transition.

### Checkpoint Control

Loss of checkpoint control, which defines the boundaries of the phases of the cell cycle ( Fig. 11-5), is common during malignant transformation. Progression through the cell cycle is tightly regulated by cyclin-dependent kinases (cdks), and their inhibitors p16 $^{INK4a}$, p21$^{WAF/CIP-1}$, and p27$^{KIP1}$ (Fig. 11-5). Controlled progression through the cell cycle is modulated by checkpoint control. [179] The transition through the phases of the cell cycle is controlled by the orderly phosphorylation and proteolysis of these cell cycle regulatory proteins. [180] The cdks are catalytic subunits that complex with cyclins to form holoenzymes that are activated by phosphorylation. [181] These kinases control cell cycle progression through protein phosphorylation events. The cyclins are proteins that are necessary for activation of cdks. During malignant transformation, progressive loss of cdk inhibitors and overexpression of cyclins leads to a growth and proliferation advantage for the malignant cell. The D cyclins (cycD), the retinoblastoma (Rb) protein, and p53 protein are often mutated in malignancy and regulate $G_1$/S progression. [178,182] Blocking of cell cycle progression with cdk inhibitors should lead to growth arrest or may result in apoptosis ( Fig. 11-5). [183,184 and 185]

To move from $G_1$ to S the tumor cell must pass the restriction point in $G_1$, the $G_1$ checkpoint. To traverse the checkpoint requires the phosphorylation of the Rb protein by cdks and the disassociation of the E2F transcription factor from the Rb protein complex. With the release of phosphorylated Rb, E2F facilitates progression by initiating the transcription of the genes important for progression through $G_1$ to S phases. [186,187 and 188]

### $G_1$ Phase and $G_1$/S Transition

CycDs associate with cdks 4 and 6 to traverse $G_1$. CycDs are synthesized in response to mitogenic activation. CycD and cdks 4 and 6 associate to form a complex that promotes the $G_1$/S transition facilitated by cdk2. [189,190] The CIP/KIP protein family inhibits cdks 4 and 6 activity (Fig. 11-5). The cycD/cdk complex phosphorylates Rb and relieves the repression of transcription by the Rb/E2F complex and the sequestration of the CIP/KIP proteins leading to activation of cycE/cdk2. CycE and cdk2 form a complex and facilitate the transition from late $G_1$ to S. Rb phosphorylation also allows E2F to activate transcription of S phase entry genes. When cycD is released and the CIP/KIP family of proteins is redistributed to cdk2, $G_1$ arrest occurs. [189]

Disruption of the $G_1$ checkpoint through loss of Rb or p16 or overexpression of cycD is universally present in human malignancies [191]; thus, restoration of the $G_1$ checkpoint is a rational therapeutic strategy. A number of investigational agents appear to act on Rb phosphorylation by cdk4. [180] Down-regulation of cycD reduces cdk4-mediated Rb phosphorylation. Agents that lead to cycD down-regulation include the retinoids, rapamycin and its ester analog CCI-779, flavopiridol, and the antiestrogens. 17-Allylamino-geldanamycin destabilizes cdk4. [180] Rb phosphorylation mediated by cdk4 is blocked by the inhibitory protein, p16 $^{INK4a}$. Hypomethylating or demethylating agents or histone deacetylase (HDAC) inhibitors that are discussed later can reactivate p16 $^{INK4a}$. [192] An alternative to reactivation of p16$^{INK4a}$ is the replacement of this cdk4 inhibitor with p16$^{INK4a}$ synthetic peptides [193] or p16$^{INK4a}$-expressing recombinant adenovirus technology. [194]

The specific cdk inhibitors currently under investigation all competitively inhibit the adenosine triphosphate (ATP)-binding pocket of the kinase. These agents are small molecules that are structurally dissimilar. The cdk inhibitors are reviewed and classified in several extensive reviews [181,195,196 and 197] Novel classes of cdk inhibitors have been generated using combinatorial libraries and structure-based design. [180] The direct inhibitors include purines, purine-like analogs, natural products, synthetics, and peptides [180,181,195,197] such as olomoucine and UCN-01 (Table 11-4). The indirect inhibitors include agents that decrease cycD levels, increase endogenous cdk inhibitors, and alter checkpoint control and other agents, such as rapamycin analog (CCI779), butyrates, and UCN-01. Analogs of flavopiridol and olomoucine cause cell cycle arrest in $G_1$ and $G_2$ as well as inhibit tumor cell growth *in vitro*. [198]

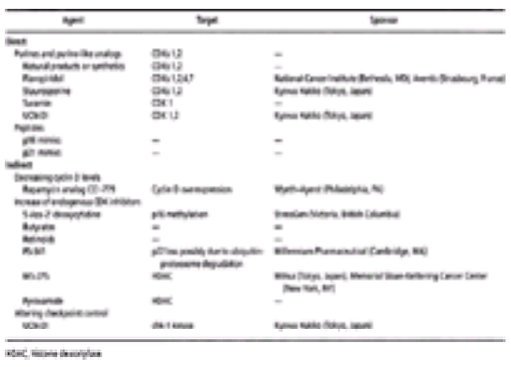


**TABLE 11-4. CYCLIN-DEPENDENT KINASE (CDK) AND CELL CYCLE MODULATORS**

### *Flavopiridol: A Cyclin-Dependent Kinase Inhibitor*

#### Background

Flavopiridol, another cdk inhibitor, is a nonchlorinated flavonoid derived from a plant alkaloid. Flavopiridol inhibits all cdks examined to date at concentrations of 100 to 300 nM. [212,213,214 and 215] In addition to inhibiting cdks, flavopiridol also decreases levels of cycD1, [216] which could contribute to $G_1$ arrest. *In vitro* testing revealed growth inhibition with $G_1$ or $G_2$ arrest in a range of cell types, [212,215] with differentiation being observed in some cases. [217] Flavopiridol induces apoptosis in some cell lines, including breast cancer, [218] chronic lymphocytic leukemia, [219] and prostate cancer. [220] Hypoxia-induced vascular endothelial growth factor (VEGF) mRNA and protein expression can be blocked by flavopiridol, and flavopiridol can induce apoptosis in endothelial cells, suggesting the potential for antiangiogenic activity. [221,222 and 223] EGFR tyrosine kinase [224] and protein kinase A (PKA) inhibition [181,224] were also noted with flavopiridol treatment.

Flavopiridol demonstrates sequence-dependent, additive, or synergistic activity with a number of chemotherapy agents, with greatest activity when the chemotherapy

agents are administered before flavopiridol.[225,226] *In vivo* studies using schedules that approximate continuous exposure demonstrated growth inhibition against several cancer cell lines.[224] The use of intermittent bolus schedules led to tumor regressions using head and neck,[227] prostate,[228] and lymphoid malignancy cell lines.[229]

### Clinical Experience

Human clinical trials of flavopiridol in adults have explored a 72-hour continuous infusion, given every 2 weeks.[230,231] Concentrations of flavopiridol that inhibit cdk activity, 300 to 500 nM, were attained. The recommended phase II dose on this schedule was 50 mg/m$^2$/day for 72 hours. In these studies the DLT was secretory diarrhea, treatable with cholestyramine and loperamide, which allowed further dose-escalation safely. The DLTs at this dose level were reversible hypotension, a proinflammatory syndrome with fever, fatigue, tumor pain, and modulation of acute phase reactants.[231] Patients with gastric cancer and renal cancer sustained a complete response and and a partial response, respectively. Minor responses were seen in NHL, colon cancer, and renal cancer. Most of the responses reported on this clinical trial were consistent with prolonged stable disease for 6 months to 1 year, suggestive of cytostatic activity.

Other schedules of flavopiridol administration have also been evaluated. Based on preclinical studies showing regressions in NHL human xenografts with bolus flavopiridol,[229] 1-hour daily infusion schedules were studied in an effort to achieve the micromolar concentrations associated with tumor regression preclinically. The MTDs on 1-, 3-, and 5-day every 21 day schedules were 37.5, 50.0, and 62.5 mg/m$^2$/day, respectively.[230] Micromolar concentrations were achieved at the highest doses, but only for brief periods. Other schedules and combinations of flavopiridol with cytotoxic chemotherapy are currently being evaluated in phase I clinical settings in both adults and children. Phase II studies are being performed in chronic lymphocytic leukemia, NSCLC, NHL, and colon, prostate, gastric, head and neck, and renal cancer. Further studies will address the pharmacodynamic parameters to best evaluate cdk inhibition and clinical trial design related to the evaluation of stable disease.

### Pediatric Applications

Flavopiridol is of potential interest in pediatric cancers primarily based on the ability of bolus schedules achieving higher plasma levels to induce apoptosis in leukemia and lymphoma xenograft models.[229] A pediatric phase I trial of flavopiridol using a daily × 3 infusion schedule is planned.

UCN-01 is of potential interest for T-ALL, based on results showing that T-ALL cells with p16 inactivation are especially sensitive to UCN-01.[232] Whereas T-ALL cells with intact p16 have an inhibitory concentration ($IC_{50}$) of more than 200 nM, cells with inactivated p16 have $IC_{50}$ values less than 50 nM. UCN-01 may also have applicability in potentiating the activity of standard chemotherapy regimens by its abrogation of $G_2$/M and S phase checkpoints.

## Translation of Proteins Required for Cell Cycle Progression and Proliferation

### *Rapamycin (Sirolimus) and CCI-779: Inhibitors of $G_1$/S Phase Transition and Protein Synthesis*

### Background

The Mammalian Target of Rapamycin (mToR) (sirolimus) and CCI-779, an ester of rapamycin (Table 11-4), act through inhibition of the mammalian target of rapamycin (mTOR) kinase, which results in diminished translation of proteins essential for proliferation.[233] Rapamycin also induces increased turnover of cycD1 mRNA and protein, which could contribute to its antiproliferative activity.[234,235] Rapamycin inhibits mTOR after first binding to the FK506 binding protein 12 (FKBP12) (Fig. 11-4). mTOR is a downstream component of the PI3'K/Akt signaling pathway that is activated by mitogenic growth factors, and mTOR is phosphorylated and activated by Akt.[236] Activation of mTOR leads to increased activity for two proteins that control the translation of mRNAs for proteins necessary for cell proliferation: (a) p70 S6 kinase ($p70^{s6k}$), which controls translation of a set of mRNAs encoding ribosomal proteins and translation elongation factors[237]; and (b) eukaryotic initiation factor (eIF)-4E, which is needed for translation of mRNAs for a set of transcription factors and other proteins involved in cell proliferation.[238] Activation of eIF-4E by mitogenic stimuli requires mTOR-mediated phosphorylation of eIF-4E binding protein (4EBP-1),[239,240] which leads to its dissociation from eIF-4E and to activation of eIF-4E.

### Clinical Experience

Rapamycin has been studied for its immunosuppressive effects in the allograft transplant setting.[241] In a randomized clinical trial for patients with renal allografts receiving cyclosporine and prednisone, those patients also receiving rapamycin (2 or 5 mg /day by mouth) had decreased occurrence and severity of biopsy-confirmed acute rejection episodes compared to patients receiving azathioprine.[242] Toxicities associated with rapamycin treatment included hyperlipidemia, arthralgias, anemia, and thrombocytopenia.[242] Trough concentrations of rapamycin greater than approximately 15 nM appear to be associated with greater risk for both thrombocytopenia and hyperlipidemia.[243] These concentrations exceed those required to inhibit $p70^{s6k}$ phosphorylation in cancer cells *in vitro*.[235,244,245] SDZ RAD, an orally administered rapamycin analog, is also under clinical evaluation as an immunosuppressive agent for organ transplant patients.[246,247]

Clinical trials of CCI-779 investigating a once-weekly and a daily × 5 schedule have studied dose ranges of 7.5 to 220 mg/m$^2$/week or 0.75 to 19.1 mg/m$^2$/day, respectively.[248,249] Toxicity has been generally mild, with skin and mucosal reactions such as dryness, urticaria, eczema-like lesions, aseptic folliculitis, and mucositis.[248,249] Hypertriglyceridemia has also been observed, and heavily pretreated patients have required dose reduction because of grade 3 thrombocytopenia.[248] Partial responses occurred in a patient with NSCLC on the daily × 5 schedule and in patients with renal cell carcinoma, breast cancer, and a neuroendocrine tumor on the weekly schedule. This antitumor activity occurred over a range of doses on both schedules. These findings suggest that a biologically active dose of CCI-779 (e.g., as determined by *in vivo* inhibition of $p70^{s6k}$)[250] below a dose with significant toxicity may be sufficient for maximum antitumor activity.

### Pediatric Applications

Interest in rapamycin and CCI-779 for pediatric cancers derives from preclinical studies showing activity of rapamycin against pediatric tumors such as rhabdomyosarcoma,[245,251,252] osteosarcoma,[253] medulloblastoma,[254] and high-grade glioma.[255,256] In addition, the rapamycin analog SDZ RAD inhibited growth of posttransplant lymphoproliferative disorder–like B-lymphoblastoid cell lines both *in vitro* and *in vivo*.[257] In contrast to results obtained in most adult cancers, rapamycin induces apoptosis in some rhabdomyosarcoma, medulloblastoma, and posttransplant lymphoproliferative disorder–like B-lymphoblastoid cell lines.[252,258] Exogenous IGF-1 can protect rhabdomyosarcoma cells against apoptosis.[245] Because GLI-transformed cells are sensitive to rapamycin,[259] pediatric tumors with an activated Hedgehog signaling pathway (in which GLI is a downstream mediator) may be appropriate targets for rapamycin evaluation. The Hedgehog pathway is activated in some cases of medulloblastoma and rhabdomyosarcoma.[259,260] In terms of combining CCI-779 with chemotherapy agents, some studies have shown a potentiating effect for rapamycin when combined with chemotherapy agents,[261,262] whereas other studies have shown either no effect or mild antagonism.[263,264] Thus, it is difficult to predict the eventual utility of combining these agents with chemotherapy.

### S Phase Events and Progression

Alterations in the Rb pathway are common in malignancy and result in persistent E2F activation and decreased cycA-cdk2 phosphorylation events that may not affect normal cells and may result in tumor cells susceptible to agents that inhibit cycA-cdk2. The end result of cycA-cdk2 inhibition may be tumor cell apoptosis. E2F has a critical role in cell cycle progression past the $G_1$ restriction point and then into S phase. E2F forms heterodimers with the DP family of proteins following Rb phosphorylation and subsequent release of E2F. The E2F-DP heterodimers transcriptionally activate the genes that encode S phase proteins transiently. CycA-cdk2 deactivates E2F to properly modulate S phase progression. The E2F-cycA-cdk2 complexes exhibit the kinase activity leading to E2F-1-DP-1 phosphorylation.[265] E2F-1 binding to DNA is suppressed by phosphorylated E2F-1-DP-1, thus modulating the expression of S phase regulatory proteins. If E2F is not deactivated by cdcA-cdk2, S phase arrest occurs and apoptosis follows.[265] Theoretically, the combination of a cdk inhibitor, flavopiridol, with S phase–specific chemotherapeutic agents would enhance the activity of this cytostatic agent. Activity should be additive or synergistic when flavopiridol is combined with cytotoxics such as cisplatinum, topoisomerase I and II inhibitors, and alkylators.[225,226,266]

### $G_2$ Checkpoint and $G_2$/M Transition

$G_2$ checkpoint inhibition plays a central role in sensitization to radiation and chemotherapy. Because the checkpoint continues to be regulated by cdc25C in the face of p53 deficiency, developing agents that modulate cdc2 phosphorylation through this bispecific phosphatase may be an effective strategy for tumor sensitization. DNA damage induces a series of events aimed at maintaining cdc2 in a phosphorylated state to block replication of the genome before mitosis by maintaining $G_2$ arrest. This cellular response provides sufficient time for both repairing DNA as well as preventing cellular replication of damaged cells that may lead to apoptosis. The investigational agent UCN-01 abrogates the $G_2$ arrest after treatment with either radiation or chemotherapy, particularly in p53-deficient cells. Other activities of UCN-01 include inhibition of cdc25C phosphorylation by chk-1, [267] cell cycle arrest in tumors expressing wild-type Rb, [180] and abrogation of the $G_2$ checkpoint after treatment with cisplatin or camptothecin. [267]

#### *UCN-01: A Serine/Threonine Kinase Inhibitor*

#### Background

UCN-01 is a 7-hydroxy staurosporine analog that inhibits serine/threonine kinases in a concentration-dependent manner. [181] Although UCN-01 is a potent protein kinase C -a, -b, and -g inhibitor, this is not the mechanism of its antitumor effect [199]; nor is UCN-01 a highly specific cdk inhibitor. UCN-01 arrests tumor cells in $G_1$ and blocks $G_2$/M checkpoint function through the Chk-1 kinase. [200,201] Inhibition of Chk-1 kinase by UCN-01 leads to inappropriate activation of Cdc2 and progression into mitosis. UCN-01 also induces $p21^{WAF/CIP-1}$ and $p27^{KIP1}$ cdk inhibitory proteins [202] that cause $G_1$ arrest by dephosphorylated Rb accumulation and decreased expression of cycA. UCN-01 also abrogates the S phase and $G_2$/M checkpoints that normally allow time for DNA repair before either DNA synthesis or mitosis, respectively. [203,204] and [205] Abrogation of S and $G_2$/M phase checkpoints enhance the cytotoxicity of DNA-damaging agents such as cisplatin. Cells with excessively damaged DNA proceed prematurely into S phase or mitosis and subsequently undergo apoptosis. UCN-01 induces DNA damage that results in the activation of caspase and serine protease(s) pathways. UCN-01 synergizes with a variety of chemotherapeutic agents with DNA-damaging effects, including cisplatin, [206] topoisomerase inhibitors, [204] Ara-C, [207] and mitomycin C, possibly by abrogating $G_2$ checkpoint function, preventing DNA repair. [208]

#### Clinical Experience

The pharmacokinetic data, from phase I studies of UCN-01 using a 72-hour continuous intravenous (i.v.) infusion every 2 weeks in the United States or a 3-hour schedule every 3 weeks in Japan, demonstrated that UCN-01 had a prolonged half-life of several weeks. The volume of distribution was very small at steady state and the systemic clearance was prolonged. [209] The delayed clearance and subsequent prolonged exposure to UCN-01 was the result of the tight binding of UCN-01 to human a-1 acid glycoproteins. [210] The U.S. study was modified so that the treatment interval was increased from 2 to 4 weeks to accommodate the prolonged half-life. The infusion time for UCN-01 was decreased to 36 hours for cycle two and all subsequent cycles to diminish agent accumulation. The highest dose administered was 53 mg/m$^2$/24 hours for 72 hours in cycle one and 36 hours every 4 weeks using the modified schedule. Using salivary concentration of UCN-01 as a surrogate for free plasma concentrations, free concentrations of UCN-01 in the salivary gland were 45 nM (range, 29 to 182 nM); these levels were sufficient to abrogate the $G_2$ checkpoint. DLTs included hyperglycemia, lactic acidosis, nausea and vomiting, and transient hypoxia with minimal or no symptoms and self-limited elevations in hepatic transaminases. Asymptomatic and transient hypotension, headache, anemia, fatigue, myalgia, anorexia, and fever were also observed. [209]

The pharmacokinetic data fit a two-compartment model. The median volume of the central compartment was 2.6 L (range, 0.1 to 9.1 L). The clearance and terminal half-life were 0.01406 L/hour (range, 0.001 to 0.04 L/hour) and 574 hours (range, 199 to 4,099 hours), respectively.

In the phase I trial of UCN-01, one patient with metastatic melanoma sustained a partial remission and several patients had prolonged stable disease of 6 months' duration or longer. [211] After disease progression on UCN-01, one patient received standard chemotherapy while UCN-01 plasma levels were still elevated; the patient achieved a complete response after this initial course of therapy. These findings suggest UCN-01 may potentiate the effects of standard chemotherapy. Combination studies with this agent will proceed based on preclinical studies that indicate the activity of UCN-01 is potentiated with S phase or DNA-damaging specific drugs such as cisplatin, Ara-C, fludarabine, and 5-fluorouracil (5-FU). [211]

## INHIBITION OF PROTEASOME-MEDIATED PROTEIN DEGRADATION

The primary route for endogenous nuclear and cytoplasmic protein degradation in eukaryotic cells is the ubiquitin-proteasome pathway. [268] Proteins are targeted for degradation by covalent linkage with multiple molecules of the small protein ubiquitin. Once targeted, proteins are rapidly degraded by proteasomes. Specificity in protein degradation resides in the enzymes involved in coupling ubiquitin to proteins. [268] Proteasome-regulated processes include cell cycle progression, transcription factor activation, apoptosis, angiogenesis, and the regulation of proliferation. [268,269] and [270]

The 26S proteasome [271,272] is critical for proteolysis of ubiquitinated proteins. The 20S proteasome core is a cylinderlike complex with four stacked rings with seven related subunits. [273] The proteolytically active sites are found on the inner surface. [274] The alpha subunits may regulate access to the inner core that is active in proteolysis. The 20S proteosome has less proteolytic activity *in vitro* than does the full 26S proteosome complex. The 26S proteosome includes the 19S regulators. Some of the 19S regulators contain adenosine triphosphatases that facilitate degradation of substrate proteins by proteosome-mediated protein identification, unfolding, and transportation into the 20S complex. [274] The energy-dependent degradation of proteins by the proteasome results in small peptides ranging in size from 4 to 25 amino acid residues. The beta subunit of the proteasome has chymotrypsin- and trypsin-type activity, as well as peptidylglutamyl peptide hydrolytic activity. Naturally occurring and synthetic inhibitors of the proteasome have been identified, [275] including the *Streptomyces* metabolite lactacystin [276] and peptide aldehydes such as MG-132. [277]

#### PS-341: An Inhibitor of Proteasome-Mediated Protein Degradation

#### *Background*

PS-341 is a dipeptidyl (L-phenylalanine-L-leucine) boronic acid derivative (Table 11-4) that is a potent (Ki approximately 0.6 nM) and selective proteasome inhibitor. [278,279] The inhibitor selectively targets the chymotryptic activity of the proteasome and has minimal activity against other serine and cysteine proteases. As determined by the National Cancer Institute *in vitro* screen, PS-341 has substantial cytotoxicity against a broad range of human tumor cells and has a unique pattern of activity suggesting a distinctive mechanism of action. [279] PS-341 induces apoptosis at nanomolar concentrations against some leukemia cell lines, an effect that does not require p53. [280] It also induces apoptosis in multiple myeloma cells *in vitro*. [281] PS-341 slowed tumor growth in prostate cancer, lung cancer, and murine mammary carcinoma *in vivo* models. [279,282] PS-341 and other proteasome inhibitors have been tested *in vitro* and *in vivo* in combination with cytotoxic agents, [282,283] and [284] in combination with radiation, [282,285] and in combination with signal transduction pathway modulators. [286] The combinations have generally been additive; however, sequencing and dose issues were not fully addressed in these experiments. The basis for the antitumor activity of PS-341 and other proteasome inhibitors is not well understood. The anticancer activity of PS-341 could result from a number of biological sequelae of proteasome inhibition, including inhibition of NF-kB activation; p53, $p21^{WAF/CIP-1}$, and $p27^{KIP1}$ stabilization; and inhibition of angiogenesis. [280,287,288] and [289]

Apoptosis, angiogenesis inhibition, cellular adhesion molecule expression, and signal transduction modulation are linked to the antineoplastic effects of PS-341. PS-341 treatment stabilizes p53 and modulates its downstream effectors, p21 and mdm2, in addition to the effectors Rb, bcl-2, and stress kinases. Increases in p21 create a basis for cdk inhibition and cell cycle arrest. Prolonged (12-hour) exposure to PS-341 results in S phase and $G_2$/M blockade. [290] After PS-341 treatment, prostate and pancreatic tumors orthotopically implanted in nude mice demonstrated a decrease in tumor cell proliferation and viability associated with decreased VEGF expression and microvessel density. [291] PS-341 inhibits activation of the NF-kB signaling pathway by interfering with IkB proteolysis. [292,293] Activation of the NF-kB pathway enhances cellular survival signals. NF-kB signaling regulates the expression of VEGF and cellular adhesion molecules. Cytotoxic agents induce NF-kB, which may result in drug resistance through control of cell survival pathways. [294] The role these target molecules play in the antitumor effects of PS-341 is unknown.

### *Clinical Experience*

The first phase I trial of PS-341 evaluated weekly i.v. bolus administration for 4 weeks over a 6-week period. [295] A preliminary report described no significant toxicity for patients treated at doses from 0.13 to 1.60 mg/m$^2$. Other phase I studies have evaluated PS-341 given twice weekly every other week for 2 weeks of a 3-week cycle[296,297] or twice weekly for 4 weeks of a 6-week schedule.[298,299] Patients on these studies received PS-341 at doses ranging from 0.13 to 1.90 mg/m$^2$. At these doses adverse events were primarily mild gastrointestinal toxicities, occasional thrombocytopenia, and sensory neuropathy. [297,298] Because excessive toxicity was observed in preclinical models when the proteasome inhibition exceeded 80%, phase I trials were designed to escalate to a PS-341 dose that achieved this level of proteasome inhibition in peripheral blood 1 hour after administration. [300] In peripheral blood of patients in the phase I studies, 20S proteasome was inhibited in a dose-dependent fashion, with up to 60% proteasome inhibition observed at PS-341 doses of 0.9 to 1.2 mg/m $^2$.[296,297] In terms of antitumor activity, one patient with prostate cancer achieved a partial response that lasted for 8 months, [295] and clinical activity has been observed for multiple myeloma. [299]

Because PS-341 is cleared so rapidly in plasma, routine pharmacokinetic analysis has not been helpful. The phase I studies described investigated 20S proteasome inhibition in the first cycle before treatment and 1 hour after treatment in the peripheral blood specimens. [300] Using this assay for proteasome inhibition, the maximum tolerated level of biologic inhibition is predicted to be approximately 80%. Biologic inhibition of proteasome activity will be used to determine the PS-341 dose.

### *Pediatric Applications*

In considering what clinical pathways to pursue after the pediatric phase I evaluation of PS-341, the effect of proteasome inhibition on several *in vitro* pediatric tumor models provides leads for possible pediatric clinical applications of the agent. Diminished ubiquitin-proteasome degradation of p27 $^{KIP1}$ during retinoic acid treatment of LAN-5 human neuroblastoma cell line was associated with a more than tenfold accumulation of p27 $^{KIP1}$ protein and with growth arrest.[301] Differentiation of a murine neuroblastoma cell line (Neu 2A) was induced on exposure to the proteasome inhibitor lactacystin. [302] Additionally, lactacystin blocked cell cycle progression beyond $G_1$ phase for synchronized Neu 2A cells and MG-63 human osteosarcoma cells *in vitro*.[302] Incomplete differentiation occurred with lactacystin treatment of human RD embryonal rhabdomyosarcoma cells.[303] Proteasome inhibition by lactacystin led to cellular apoptosis associated with the accumulation of ubiquitinated protein and activation of bcl-2–sensitive apoptotic pathways in the Ewing's sarcoma cell line A4573. [304] Peptidyl aldehyde proteasome inhibitor given in a single dose induced early tumor regression and a significant delay in tumor progression in a murine model of human Burkitt's lymphoma. [305]

Given the ability of NF-kB to stimulate proliferation and confer resistance to apoptosis, and given the ability of PS-341 to interfere with NF-kB signaling, pediatric tumors with activated NF-kB are also potential targets for PS-341 evaluation. Constitutive NF-kB activation is a common characteristic of childhood ALL [306] and is frequently observed in Reed-Sternberg cells from cases of Hodgkin's disease. [307] Treatment of Hodgkin's cells with the proteasome inhibitor MG-132 induced apoptosis and sensitized them to ionizing radiation. [285]

A pediatric phase I study of single-agent PS-341 has been opened. Given preclinical data suggesting a favorable interaction with other treatment approaches, including cytotoxic agents,[283,284] radiation,[282,285] and signal transduction pathway modulators,[286] the eventual utility of PS-341 in the treatment of pediatric cancers will most likely be in combination with other anticancer treatments. Preclinical data using pediatric tumor models may provide useful information for prioritizing possible combination studies.

## HEAT SHOCK PROTEIN AND INHIBITION OF CHAPERONE FUNCTION

### 17-Allylamino-17-Demethyoxygeldanamycin: An Inhibitor of Chaperone Function

### *Background*

Geldanamycin appears to exert its biologic activities primarily through interaction with heat shock protein 90 (hsp90). [308] Hsp90 is a member of the chaperone family of proteins that act in multimeric complexes to achieve and maintain appropriate folding and activation of client proteins. [309] Hsp90 contains a nucleotide-binding domain within its amino terminal. ATP binding and hydrolysis are essential for the function of hsp90. [310,311 and 312] Geldanamycin binds hsp90 at the ATP binding site and inhibits ATP binding and hydrolysis.[311,312] Geldanamycin compounds bind to hsp90 and the homologous chaperone protein Grp94, thereby disrupting the interaction between these chaperone proteins and many kinases and other molecules involved in proliferation and cell cycle control. [313,314,315,316,317,318 and 319] The physiologic consequence of geldanamycin binding to hsp90 is destabilization of hsp90 client proteins and their degradation through the ubiquitin-proteasome pathway. [320] The cell signaling pathways affected by geldanamycin include the following [309]: glucocorticoid-induced gene expression, [321] ErbB-2 signaling,[322] signal transduction pathways involving Raf,[317] signaling through Src family protein kinases,[323] p210$^{bcr-ab}$ signaling,[324] focal adhesion kinase signaling, [325] hypoxia-induced gene expression,[326,327] and T-cell activation and survival. [328] The variety of signal transduction pathways affected by geldanamycin [329,330 and 331] suggests obvious anticancer applications for the agent.

Although geldanamycin was broadly cytotoxic in the National Cancer Institute cell line screen, it was not developed further due to unacceptable preclinical toxicity and poor solubility. The first semisynthetic derivative inhibitor of the chaperone function of hsp90 is 17-allylamino-17-demethyoxygeldanamycin (17-AAG). Although this derivative does not bind as tightly to the ATP/adenosine diphosphate binding pocket, 17-AAG exhibits anti-proliferative effects similar to those of geldanamycin in human breast cancer cell lines and also decreases expression of p185 $^{erbB-2}$, Raf-1, and p53 at similar concentrations.[316] Sequence-dependent synergy was observed with paclitaxel.[332]

### *Clinical Experience*

17-AAG is being studied in phase I clinical trials that examine a daily schedule for 5 days every 3 weeks and a weekly schedule. The clinical trials are in early implementation stages and only limited pharmacokinetic data are available. Peak plasma concentrations range from 0.53 to 1.30 µM at dose levels 10, 14, and 20 mg/m$^2$ daily for 5 days every 3 weeks. The alpha– and beta–half-lives were 0.10 to 0.19 hours and 1.8 to 3.7 hours, respectively. The volume of distribution ranged from 11 to 182 L at steady state concentrations. No agent accumulation was observed during the 5 days of administration. [333]

### *Pediatric Applications*

Because of the diverse range of signaling pathways inhibited by geldanamycin, a number of pediatric tumors could be sensitive to the cytotoxic effects of the agent. An early report on geldanamycin demonstrated its activity against primitive neuroectodermal tumor cell lines, [334] suggesting potential applicability against the Ewing's family of tumors. The ability of geldanamycin to down-regulate ErbB-2 suggests potential applicability for medulloblastoma and osteosarcoma, two tumors known to express ErbB-2.[335,336 and 337] With further testing of geldanamycin in pediatric preclinical models, other tumors may be identified as potential targets for clinical evaluation of 17-AAG or other agents that inhibit hsp90 function.

## ANGIOGENESIS, MALIGNANT TRANSFORMATION, INVASION, AND METASTASIS

The search for molecular solutions targeted to cancer has intensified the study of malignant transformation, tumor invasion, and metastasis. Neoplastic transformation involves molecular and cellular processes that are intimately related to the formation of new capillary blood vessels, or angiogenesis. The earliest steps in the development of malignancy involve tumor initiation, promotion and transformation from a normal cell to a carcinoma *in situ* (Fig. 11-6). The next step in this sequence involves the angiogenic switch during which avascular tumors recruit their blood supply. [338,339] This sequence of events is followed by the recruitment of circulating endothelial cells to the tumor focus of angiogenesis. [340,341 and 342] Some tumors will stimulate fibroblasts in the tumor microenvironment to express VEGF, thus contributing to the change to an angiogenic phenotype. [343] Finally, as the tumor cells extravasate from blood vessels they induce endothelial cell proliferation and promote neovascularization. In addition, once tumors have established an angiogenic phenotype, the integrity of the basement membrane is compromised following increased expression of matrix metalloproteinase 2 (MMP-2; Gelatinase A), MMP-9 (Gelatinase B), collagenase IV, and membrane-type metalloproteinase 14 (MT1-MMP).[344] Tumor cells then reenter the circulation through intravasation of blood vessels and lymphatics via the extracellular matrix (ECM), extravasate distally,

and establish growth in a new microenvironment.[345]

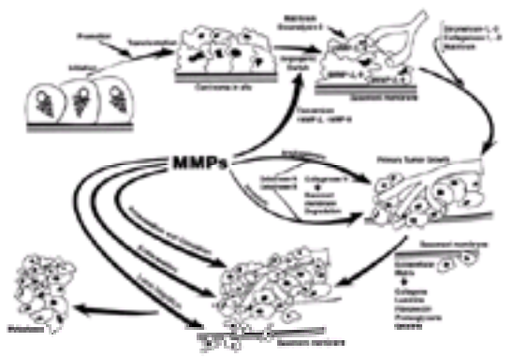



**FIGURE 11-6.** The role of matrix metalloproteinase (MMP) in tumor progression and metastases. The MMPs play a pivotal role in the angiogenic switch, angiogenesis, invasion, intravasation, and extravasation of tumor cells, local migration, and tumor metastasis, thereby making them suitable targets for control of tumor progression and metastases.

### Blockade of Matrix Breakdown as a Target: Matrix Metalloproteinase Inhibitors

The MMPs and their endogenous inhibitors, the tissue-localized tissue inhibitors of metalloproteinases (TIMPs), [346] modulate tumor cell invasion and metastasis through their interactions with the ECM, a metabolically active tissue.[345] ECM is composed of type IV collagen, laminin, entactin, proteoglycans, and glycosaminoglycans.[347] The MMPs are members of a family of enzymes that are regulated by growth factors, cytokines, and oncogenes.[348] The membrane-type MMPs require cleavage by other MMPs or proteases to release the active enzyme from the membrane.[349] In response to tumor cell invasion, host connective tissue cells, such as fibroblasts and inflammatory cells, induce the expression of MMPs.[350],[351] and [352] MMPs are expressed in a vast array of human tumors, making them unique targets for cancer therapy.[353] Because MMPs facilitate the processes of invasion, angiogenesis, and metastasis, MMP inhibitors may require chronic administration in a minimal disease setting to be most effective.

#### *BMS-275291: A Membrane-Type Metalloproteinase 14*

BMS-275291 (D2163) is an MMPI with $IC_{50}$ values in the range of 10–25 nM against MMP –1, -2, -8, and –9 and as well has activity against MMP-14 (MT-1 MMP). MT-1 MMP is involved in the activation of MMP-2. This relatively selective MMPI is only active in a micromolar range against TNF-a converting enzyme (TACE) and may not be associated with dose-limiting joint problems (see discussion under marimastat below). In metastatic models, progression is slowed by 60% to 90%. A phase I clinical trial is evaluating the potential biologic activity of BMS-275291 using the skin biopsy healing assay at doses ranging from 600 to 2,400 mg. [354]

#### *COL-3: An Enhancer of Matrix Metalloproteinase Degradation*

**Background**

COL-3 is a tetracycline analog, 4-dedimethylamino sancycline. By formulating OL-3 without the dimethyl amino group at the C4 position, its antimicrobial activity is abolished. COL-3 has anti-MMP activity via decreased expression of MMP-2 and MMP-9. Diminished MMP-2 expression may inhibit tumor growth and metastases. Inhibition of MMP-2 is not achievable *in vivo* (Ki 148 µM).[355] On matrigel invasion assays,[356],[357] COL-3 demonstrated 60% to 90% inhibition of tumor cell invasion. Inhibition of MMP expression has been demonstrated *in vitro* using human colon cancer and breast cancer cell lines also.

**Clinical Experience**

A phase I study of COL-3 demonstrated acceptable toxicity using doses ranging from 36 to 98 $mg/m^2$ in adults with advanced cancer. Toxicities included photosensitivity (grade 2 or 3), anemia (grades 3 to 4), fatigue (grades 2 to 3), and minimal nausea or vomiting (grades 1 to 2). The $C_{max}$ ranged from 1.0 to 2.6 µg/mL, even at doses ranging from 70 to 98 $mg/m^2$. The elimination half-life and renal clearance were 58 hours (range 24 to 140 hours) and 10 mL/minute, respectively. Plasma VEGF level did not change with COL-3 treatment; MMP-2 levels decreased slightly in the study subjects.

#### Marimastat: Matrix Metalloproteinase Inhibitor of Matrix Metalloproteinases 1, 2, 3, 7, 9, and 12

Marimastat inhibits MMP-1, -2, -3, -7, -9, and -12. Marimastat is an orally administered agent. Pharmacokinetic evaluation of this agent demonstrated trough plasma concentrations ranging from 30 ng/mL [5 mg twice daily (b.i.d.)] to 400 ng/mL (75 mg b.i.d.).[358] The elimination half-life is 8 to 12 hours [358] in healthy volunteers. Cancer patients have a shorter elimination half-life (4 to 5 hours). [359] The agent is excreted renally (40%) and is metabolized (60%). [358] Phase I trials determined that extended administration is feasible, but unusual joint problems limited chronic administration of marimastat at the upper dose levels. Musculoskeletal problems developed in 30% of patients at doses of 10 mg bid twice a day administered over 3 to 5 months [358] and are associated with prolonged administration of other hydroxamic acid–type MMPIs. The musculoskeletal problems include tendonitis that manifests as joint stiffness and pain in the hands, arms, and shoulder that resolve with interruption of therapy and dose modification. Half of the patients (6 of 12) in a study of patients with advanced gastric cancer had either a frozen shoulder or Dupuytren's-like disease.[360],[364]

Marimastat has completed phase II trials in lung, pancreas, colon, ovarian, prostate, [361],[362] and gastric cancer.[363] There were no objective responses in these studies. Some patients with pancreatic and colorectal cancer had their disease status monitored by the serum markers CA19-9 and CEA, [361],[362] respectively. Some of these patients did show a decreased *rate of increase* of these serum markers, pretreatment versus on-study rate of increase, but this may only represent regression toward the mean.

Marimastat has also been studied in phase III trials for advanced gastric cancer using 10 mg b.i.d., versus placebo, and for pancreatic cancer studying marimastat, 10 mg b.i.d., versus gemcitabine. Neither study showed a survival advantage for marimastat as a single agent. [358] Phase III studies with marimastat are proceeding in advanced breast cancer, small cell carcinoma of the lung, NSCLC, and glioblastoma. Marimastat has been combined with doxorubicin and cyclophosphamide, [365] carboplatin,[366] carboplatin and paclitaxel,[367] and gemcitabine or 5-FU.[368],[369]

#### *AG-3340 (Prinomastat): Inhibitor of Matrix Metalloproteinases 2, 3, 9, and 13*

**Background**

Prinomastat was rationally designed using protein structure based–modeling of human MMP active sites. Prinomastat inhibits MMP-2, -3, -9, and -13 but not MMP-1. Prinomastat is an orally bioavailable MMPI with an $IC_{50}$ of 8.3 µM.[370] Its half-life is short, 3 hours; it is 70% protein bound. Dose and schedule were predicted on preclinical models of growth inhibition that predicted that the maintenance of plasma concentrations above 1 ng/mL rather than total daily dose would be clinically effective.[371] Prinomastat given to nude mice with human colon tumors (COLO-320DM) every 6 hours at a dose of 6.25 mg/kg resulted in a maximal growth inhibition of 74% ($p$ <.05). When Prinomastat was administered every 12 (12.5 mg b.i.d.) or 24 (25 mg daily) hours, there was no growth inhibition in the COLO-320DM nude mouse model.

Combination of Prinomastat with other agents was evaluated preclinically. Prinomastat combined with carboplatin increased survival in an orthotopic nude rat model of primary and metastatic human lung cancer.[372],[373] An oral dose of 100 mg/kg/dose of Prinomastat was compared to an intraperitoneal dose of 10 or 20 mg/kg/dose of

carboplatin; neither agent improved overall survival in the orthotopic nude rat model. Prolongation of overall survival ( $p$ <.03) was observed when prinomastat combined with carboplatin was compared to carboplatin alone, 41.6 ± 2.5 days versus 35.7 ± 2.7 days.

### Clinical Experience

Prinomastat was studied at doses between 5 and 100 mg twice daily.[374] Minor regressions were reported in 3 of 47 patients and stable disease lasting from 4 to 10 months was reported in 12 of 47 patients. Interruption of therapy and dose modification were used to manage the musculoskeletal adverse events. Prinomastat was combined with paclitaxel (200 mg/m$^2$) and carboplatin (AUC = 6) in a phase I combination study. The expected chemotherapy-associated adverse events included rash, dysgeusia, and myalgia. Other combination studies examined Prinomastat with mitoxantrone and prednisone.[375] Phase III studies in patients with NSCLC and advanced hormone-refractory prostate cancer are evaluating 15 mg twice-daily dosing in conjunction with chemotherapy.

## ANGIOGENESIS

Angiogenesis is stimulated by VEGF receptor signaling pathways ( Fig. 11-7). These signaling pathways enhance cellular survival through Akt/PKB, stimulate proliferation and enhance vasopermeability by PLC and DAG, activate transcription through Ras [428] using the mitogen-activated protein kinases and c-*Jun*,[429] and cause cell migration and cytoskeletal rearrangement via FAK.[430,431] The preceding molecular events all lead to angiogenesis. Vascular endothelial cell receptors primarily involved in angiogenesis are Flt-1, an *fms*-like kinase, a 180-kd tyrosine kinase, and a 200-kd human kinase KDR (kinase insert domain-containing receptor), homologue of the mouse Flk-1.[432] VEGF expression increases in the presence of hypoxia and tumor necrosis.[433,434,435 and 436]

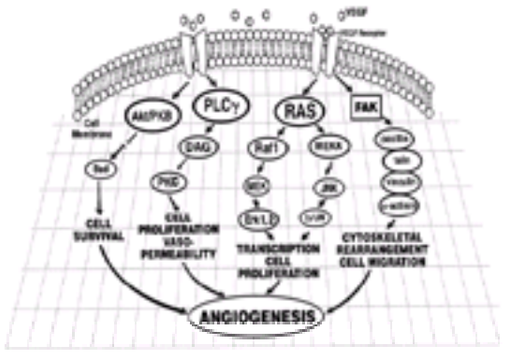



**FIGURE 11-7.** Angiogenesis activation by vascular endothelial growth factor (VEGF) receptor–signaling pathways. Activation of the Akt/PKB, PLCg, Ras, and FAK pathways lead to cell survival, cell proliferation and vasopermeability, transcription and cell proliferation, and cytoskeletal rearrangement and cell migration, respectively. Each of these events contributes to the angiogenic phenotype of the tumor cell.

Tumor cells are also able to inhibit endogenous angiogenesis at a distance. Stimulators and inhibitors modulate the process of angiogenesis and define the switch to an angiogenic phenotype.[437,438,439 and 440] Folkman's hypothesis that tumors may stimulate neovascularization locally and inhibit this process distally was borne out by the discovery of endostatin,[441,442 and 443] angiostatin,[427,444,445,446 and 447] angiopoietins,[448,449,450,451,452,453,454,455,456 and 457] VEGF,[458,459,460,461 and 462] and other molecules.[432,463,464]

Targeting the inhibition of angiogenesis as a therapeutic strategy is challenging because of the multifactorial nature of this intricate process. Functionally, the agents that specifically inhibit angiogenesis ( Table 11-5) block matrix breakdown, inhibit endothelial cells directly, block activators of angiogenesis, inhibit endothelial-specific integrin/survival signaling, or have other mechanisms of action.

| Agent | Mechanism of action | Sponsor |
|---|---|---|
| **Agents that block matrix breakdown** | | |
| Marimastat | Synthetic inhibitor of MMPs | British Biotech (Annapolis, MD) |
| Prinomastat (AG 3340) | Synthetic MMP inhibitor | Agouron (Salella, LA) |
| COL-3 | Synthetic MMP inhibitor: tetracycline derivative | Collagenex (Newtown, PA) |
| Neovastat | Naturally occurring MMP inhibitor; inhibits VEGF activity | Aetema (Sainte-Foy, Canada) |
| BMS-275291 | Synthetic MMP inhibitor | Bristol-Myers Squibb (Wallingford, CT) |
| **Agents that inhibit endothelial cells directly** | | |
| TNP-470 | Synthetic analog of fumagillin; inhibits endothelial cell growth | Tap Pharmaceuticals (Deerfield, IL) |
| Thalidomide | Blocks VEGF; inhibits angiogenesis and tumor necrosis factor pro-duction | Commercially available, approved for leprosy (Celgene Warren, NJ) |
| Squalamine | Extract from dogfish shark liver; inhibits sodium hydrogen exchanger (NHE) | Magainin Pharmaceuticals (Plymouth Meeting, PA) |
| Combretastatin A-4 prodrug | Induction of apoptosis in proliferating endothelial cells | Oxigene (Boston, MA) |
| Endostatin | Inhibition of endothelial cell function | Entremed (Rockville, MD) |
| 2-Methoxyestradiol | Inhibits cellular proliferation; inhibits tubulin function; up-regulates death receptor 5 | Entremed (Rockville, MD) |
| Angiostatin | Inhibits endothelial cell function | Entremed (Rockville, MD) |
| IL-12 | Inhibits endothelial cell function<br>Up-regulation of interferon-γ and IP-10 | Entremed (Rockville, MD)<br>Genetics Institute (Bethesda, MD) |
| **Agents that block activators of angiogenesis** | | |
| SU5416 | Blocks VEGF receptor signaling (kinase insert domain-containing receptor-specific) | Sugen (South San Francisco, CA) |
| SU6668 | Blocks VEGF-R2, FGF receptor 1, and PDGF receptor signaling | Sugen (South San Francisco, CA) |
| Interferon-α | Inhibition of basic FGF and VEGF production | Commercially available |
| Anti-VEGF antibody | Monoclonal antibody to VEGF prevents receptor ligation | National Cancer Institute (Bethesda, MD); Genentech, (San Francisco, CA) |
| **Agents that inhibit endothelial-specific integrin/survival signaling** | | |
| EMD121974 | Small molecular blocker of integrin receptor present on endothelial cell surface $\alpha_v\beta_3$, $\alpha_v\beta_5$ | Merck KGaA (Darmstadt, Germany) |
| Vitaxin | Antibody, blocks human $\alpha_v\beta_3$ | MedImmune Inc (Gaithersburg, MD) |
| **Agents with other mechanisms of action** | | |
| CAI53 | Antiviral mechanism; inhibits VEGF, stimulates IL-12 | Cytran (Kirkland, WA) |

VEGF, vascular endothelial growth factor.

**TABLE 11-5. COMPOUNDS WITH SPECIFIC ANTIANGIOGENESIS ACTIVITIES**

### Direct Inhibitors of Endothelial Cells

#### *Thalidomide : An Angiogenesis Inhibitor*

#### Background

Thalidomide is an old agent that has developed a new life as an immunomodulating agent and as an angiogenesis inhibitor ( Table 11-5). Initial attempts to license this agent in the United States were denied because of its teratogenic effects on limb development. The teratogenic effects appear to involve a species-specific hepatic metabolite.[376] Using the rabbit corneal micropocket assay, investigators have demonstrated that thalidomide inhibits both VEGF and basic fibroblast growth factor (bFGF)–stimulated angiogenesis.[377] Attribution of the antitumor activity of thalidomide to an anti-angiogenic mechanism is complicated by the multiple other biologic activities of this agent,[378] including oxidative damage to DNA,[379] inhibition of TNF-a production,[380] T-cell costimulation accompanied by increases in interleukin-2 and interferon-g production,[381] and modulation of adhesion molecule profile.[382]

#### Clinical Experience

Thalidomide is administered as a single oral dose at night. Its primary toxicities are sedation, constipation, and peripheral neuropathy. Activity for thalidomide was observed in patients with HIV-associated Kaposi's sarcoma, with 47% achieving an objective response.[383] Activity was also observed for patients with renal cancer who received low-dose thalidomide (100 mg daily), with 3 of 18 patients showing partial responses, but no responses occurred among 48 adults with other cancer types who received low-dose thalidomide.[384] Similarly, no objective responses were noted among 28 heavily pretreated breast cancer patients randomized to receive either a low dose (200 mg) or a high dose (800 mg) of thalidomide.[385] A study of thalidomide in 39 patients with relapsed high-grade gliomas demonstrated two partial responses and two minor responses and a median time to progression of only 10 weeks.[386] Thalidomide has substantial activity against multiple myeloma.[387,388 and 389] In a study of 84 patients with previously treated multiple myeloma, including those treated with high-dose chemotherapy, 32% had at least a 25% reduction in serum or urine paraprotein levels at a median time of 29 days (range, 4 days to 6 months).[389] Decrease in paraproteins was associated with a bone marrow response (a 50% reduction in the number of plasma cells) in 81% of all evaluable patients.

Thalidomide has been evaluated with a variety of chemotherapy agents in phase I/pilot studies, including carboplatin,[390] carboplatin and paclitaxel,[391]

cyclophosphamide and dexamethasone,[392] and temozolomide.[393] In general, thalidomide has been well tolerated in combination with standard chemotherapy. Three phase III trials in newly diagnosed stage III NSCLC, in multiple myeloma that has progressed following initial therapy, and in previously untreated renal cell carcinoma are evaluating whether overall survival is altered by the addition of thalidomide to standard treatment. A combination study combining thalidomide with temozolomide will be studied in children with recurrent brain tumors.

### *Combretastatin: An Endothelial Cell Interactive Agent*

#### Background

Combretastatin A4 (CA4P) is administered as a prodrug (Table 11-5) and has direct endothelial cell interactions. CA4P inhibits tubulin polymerization through its interaction with the colchicine binding site of microtubules.[394] Using a radiometric assay to measure activity, CA4P demonstrated *in vitro* activity against a variety of cell lines, including breast, colon, and lung tumor cell lines.[395] *In vivo*–selective antivascular activity has been shown in murine and rat models. This selective antivascular activity was associated with altered tumor perfusion by magnetic resonance imaging (MRI), intratumoral acidosis and hypoxia, diminished cellular energy status by magnetic resonance spectroscopy, and enhanced intratumoral necrosis.[396,397 and 398] Combretastatin is mainly active against proliferating endothelium selectively resulting in disruption of endothelial cell function.[396,399,400] Combretastatin causes apoptosis in proliferating endothelial cells[399] by inducing caspase-3 activity.[400] In murine models with established tumors, combination studies of combretastatin and cytotoxic therapies, including radiation therapy, cisplatin, or 5-FU, showed additive or synergistic activity compared to single-agent/modality treatments.[401,402 and 403]

#### Clinical Experience

Initial trials with combretastatin using three different schedules demonstrated toxicities, including shortness of breath, reversible mild cardiac ischemia thought secondary to coronary artery vasospasm, and pain at the tumor site.[404,405] Reductions in tumor blood flow demonstrated by MRI have been also been described.[405]

### *Endostatin: An Anti-Angiogenic Agent*

#### Background

Endostatin is a 20-kd protein fragment of the carboxy-terminal end of collagen XVIII (Table 11-5) that was isolated initially from supernatants of cultured murine hemangioendothelioma cells.[406] Endostatin preparations inhibit proliferation and migration of endothelial cells *in vitro* but do not affect other cell lines.[406,407] Murine endostatin induced dose-dependent regressions and prolonged growth inhibition against several murine tumors as well as human xenografts.[406] Remarkably, when tumors that had regressed were allowed to regrow to their original size, resumption of treatment with endostatin again induced complete regressions.[408] No resistance was noted following up to six cycles of regrowth and retreatment. Tumors eventually failed to regrow after multiple cycles of tumor recrudescence and treatment with endostatin, demonstrating sustained tumor dormancy.

Instability of the endostatin protein and formulation issues confounded preclinical evaluation of the agent. Initial studies were done with a precipitated form of endostatin administered as a suspension.[406,408] Recent studies have used recombinant endostatin expressed in the yeast *Pichia pastoris* system, which allows posttranslational modifications and production of soluble endostatin.[407,409] Recombinant human endostatin produced in this way inhibits the proliferation and migration of endothelial cells and induces endothelial cell apoptosis.[409] t can induce regressions *in vivo*, although the effect is not as dramatic as that initially reported using the precipitated form of murine endostatin.[408,409]

Endostatin's mechanism of action is unknown. It induces endothelial cell apoptosis *in vitro*.[410,411 and 412] Human endostatin is reported to interact with $a_5$- and $a_V$-integrins on the surface of endothelial cells.[412] Endostatin inhibits cell migration on immobilized gelatin (an $a_V b_3$ integrin ligand) but not on immobilized collagen I (a non-RGD–binding b1 integrin ligand),[412] consistent with the hypothesis of $a_5$- and $a_V$-integrins as potential targets for endostatin.

#### Clinical Experience

Phase I clinical trials with endostatin are ongoing in adult patients with advanced cancer. In one study, patients have been treated using a daily i.v. schedule at doses ranging from 15 to 180 mg/m$^2$.[413] The $C_{max}$ at the 180 mg/m$^2$ dose level was 5 µg/mL, which is approaching the target $C_{max}$ as estimated from preclinical studies of recombinant human endostatin.[414] For comparison, normal healthy controls have serum endostatin levels of approximately 15 ng/mL.[415,416] Endostatin dose escalation continues in the absence of any reported DLT. The phase I studies are evaluating tumor biopsies, noninvasive functional imaging, and pharmacokinetics to provide information for determining a biologically effective dose.[413]

### *2-Methoxyestradiol: An Estrogen Metabolite with Anti-Angiogenic Activity*

2-Methoxyestradiol (2-ME), a minor metabolite of human estradiol (Table 11-5), demonstrates anti-angiogenic activity in chick chorioallantoic membrane (CAM) model and in the corneal micropocket assay.[417,418] This relatively nontoxic orally administered agent reduced xenograft growth and tumor vascularization in murine models.[418] 2-ME inhibits lung metastases in a pancreatic cancer metastatic model[419] and has activity in breast xenograft models that are estrogen receptor negative.[417]

Induction of apoptosis in tumor and endothelial cells may in part be mediated by 2-ME binding to the colchicine site of microtubules,[420,421] leading to inhibition of proliferation. 2-ME may also induce apoptosis via expression of the death receptor 5 (DR5) gene in human umbilical vein endothelial cells and tumor cell lines regardless of the p53 status.[422] DR5 up-regulation is linked to activation of intracellular caspases in a cell type–specific fashion. Thus, 2-ME may exert its pro-apoptotic effects in various cell types by different mechanisms. Phase I clinical development of 2-ME started recently.

### *Angiostatin: An Anti-Angiogenic Agent*

Working at the level of the endothelial cell,[423] angiostatin is formed by cleavage of plasminogen and consists of kringle loops 1 to 4. Local tumor-infiltrating macrophages or the tumor may secrete elastase which cleaves plasminogen via digestion by elastase.[424] Some angiostatin preparations inhibit *in vitro* endothelial cell proliferation and *in vivo* tumor growth and induce dormancy in established tumors *in vivo*.[425] Angiostatin may induce apoptosis in proliferating endothelial cells[426]; it may also potentiate the antitumor effects of radiation therapy, in part through increasing the sensitivity of endothelial cells to radiation.[427] Development of this agent is ongoing.

### Blockade of Activators of Angiogenesis

Blockade of stimulatory factors of angiogenesis is another approach to the inhibition of angiogenesis. Angiogenesis inhibition may be accomplished by prevention of release of the factor, binding of free circulating factor, blocking binding of the factor to its receptor, or inhibiting postreceptor signaling. Basic fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) appear to be the most important stimulators of angiogenesis. Inhibition of VEGF can inhibit tumor growth and prevent metastasis in animal models. The development of small molecules that inhibit the VEGF/Flk-1/KDR (kinase insert domain-containing receptor) RTK and block signal transduction through the VEGF signaling pathway has been a major focus in new agent development.

### *SU5416: Selective Inhibitor of Vascular Endothelial Growth Factor Receptor 2 (Flk-1/KDR) Receptor Tyrosine Kinase*

#### Background

SU5416 is a synthetic molecule that is a potent and selective inhibitor of VEGF receptor 2 (Flk-1/KDR) RTK ( Table 11-2 and Table 11-5).[458] SU5416 inhibits VEGF-dependent proliferation of endothelial cells but demonstrates little effect on tumor cell growth.[465] SU5416 inhibits growth of human, rat, and murine tumor

xenografts at nontoxic doses *in vivo*.[465,466 and 467] SU5416 effects on cultured endothelial cells occur within minutes and persist longer than 3 days after washout, as compared to the short half-life (30 minutes) in rats when administered intravenously. [468] This long-lasting inhibition is attributable to the intracellular accumulation and persistence of SU5416[468] and may explain the similar level of antitumor activity for SU5416 whether administered on a twice-weekly or a daily schedule. [468]

### Clinical Experience

Phase I evaluations of SU5416 in adults explored dose levels ranging from 4.4 to 190.0 mg/m $^2$.[469] The MTD of SU5416 was 145 mg/m$^2$, with DLTs of projectile vomiting, headache, and nausea that resolved within 24 to 48 hours. Systemic exposure at the MTD achieved the AUC required for growth inhibition in animals. [469] The b-elimination half-life of SU5416 is approximately 30 minutes, and there is an approximately 70% induction of clearance of SU5416 after 1 month of twice-weekly treatments.[470] Objective responses in the phase I setting were observed for Kaposi's sarcoma, metastatic basal cell carcinoma, colorectal carcinoma, and lung cancer.[458,469,471,472] Single-agent phase II trials and studies of SU5416 in combination with chemotherapy agents are ongoing in adults. [458]

## *SU6668: Receptor Tyrosine Kinase Inhibitor That Inhibits Flk-1/KDR, Fibroblast Growth Factor Receptor 1, and Platelet-Derived Growth Factor Receptor*

### Background

SU6668 is a synthetic small molecule RTK inhibitor ( Table 11-2 and Table 11-5) that inhibits Flk-1/KDR, fibroblast growth factor receptor 1 (FGFR1), and PDGF receptor.[473] It inhibits *in vitro* proliferation of endothelial cells stimulated by FGF or VEGF, and it suppresses tumor angiogenesis *in vivo*.[473] *In vivo* testing of SU6668 revealed growth inhibition, and in some cases tumor regression, against a range of human tumors, including glioma, melanoma, and lung, colon, ovarian, and epidermoid tumor cell lines.[473] SU6668 is taken up at a slower rate than that of SU5416, and unlike SU5416, SU6668 does not persist intracellularly and does not have long-lasting activity in functional assays of VEGF inhibition following washout. [468] Studies in rats demonstrated that SU6668 is an orally bioavailable agent (approximately 50%) with a half-life of 2 to 3 hours.[474]

### Clinical Experience

A phase I trial of daily, orally administered SU6668 has been initiated in adults, with doses between 100 and 1,600 mg/m $^2$ evaluated with only moderate toxicity observed.[475]

## *rhuMAB Vascular Endothelial Growth Factor (Bevacizumab): An Inhibitor of Vascular Endothelial Growth Factor–Induced Endothelial Cell Mitogenesis*

### Background

Inhibition of VEGF signaling using an anti-VEGF monoclonal antibody blocks VEGF-induced endothelial cell mitogenesis, [476] and inhibits the *in vivo* growth of a number of human cancer cell lines, including prostate cancer,[477] glioblastoma,[478] colorectal cancer,[479] and rhabdomyosarcoma.[480] The combination of anti-VEGF antibody and chemotherapy in nude mice injected with human cancer xenografts results in an increased antitumor effect compared to either antibody or chemotherapy alone.[6]

### Clinical Experience

A phase I study evaluated bevacizumab at doses from 0.1 mg/kg to 10.0 mg/kg in adults with advanced malignancies.[481] Therapy was generally well tolerated without drug-related grade 3 to 4 or acute infusion-related toxicities, although three patients did experience tumor-related hemorrhagic events. In a second phase I study, weekly bevacizumab (3 mg/kg/dose) was given with one of three cytotoxic chemotherapy regimens (5-FU/leucovorin, carboplatin/paclitaxel, or doxorubicin). [482] The addition of bevacizumab to chemotherapy was well tolerated without apparent long-term synergistic toxicity. No antibodies to bevacizumab were detected in either study. The pharmacokinetics of bevacizumab in the single-agent study were linear for doses of greater than or equal to 1 mg/kg, with a half-life of approximately 17 days.[481] Coadministration of bevacizumab with cytotoxic chemotherapy did not appear to result in altered systemic concentrations of the cytotoxic agents. [482]

Single-agent phase II studies were conducted in women with relapsed metastatic breast cancer [483] and men with hormone-refractory prostate cancer,[484] and phase II studies combining bevacizumab with chemotherapy were conducted in adults with NSCLC[485] and metastatic colorectal cancer.[486] In the single-agent studies, objective responses were observed in patients with breast cancer[483] but not in patients with prostate cancer.[484] In the NSCLC and colorectal cancer trials with bevacizumab in combination with standard chemotherapy, there was preliminary evidence for increased response rates and prolonged time to disease progression for patients receiving bevacizumab plus chemotherapy compared to those treated with chemotherapy alone.[485,486] The most worrisome toxicities were observed in the NSCLC study, in which six subjects among 67 who received bevacizumab had life-threatening hemoptysis or hematemesis, and four of these patients died. Hemorrhage that was not life threatening was seen in the other phase I and II studies. Both venous and arterial thromboses, ranging in severity from catheter occlusion to fatal pulmonary embolus, were seen in subjects treated with bevacizumab in the colorectal cancer trial and, to a lesser extent, in the NSCLC trial.

### Inhibitors of Endothelial-Specific Integrin/Survival Signaling

Endothelial cells express multiple cell surface integrins that bind to ECM and matrix breakdown products. The vitronectin receptor $a_Vb_3$ integrin is expressed on stimulated, angiogenic endothelial cells but has limited expression on normal endothelial cells and other normal cell types. [487] A few tumor types express high levels of $a_Vb_3$ integrin, including melanoma[488] and high-grade gliomas.[489] The $a_Vb_3$ integrin binds to the Arg-Gly-Asp (RGD)-specific amino acid consensus sequence found in matrix proteins, including vitronectin, fibronectin, fibrinogen, and proteolyzed collagen. Antibodies that block a $_Vb_3$ integrin inhibit *in vivo* angiogenesis induced by basic FGF and by implanted tumors in the CAM assay.[490] Cyclic peptides that block the $a_Vb_3$ integrin RGD binding site also inhibit *in vivo* angiogenesis and caused regressions of transplanted human tumors in the CAM assay.[491] Both the monoclonal antibody strategy and the RGD cyclic peptide strategy for blocking a $_Vb_3$ integrin binding have entered clinical evaluation.

## *Vitaxin: A Humanized Monoclonal Antibody against a $_Vb_3$ Integrin*

Vitaxin is a humanized derivative of the mouse monoclonal antibody LM609 that targets the human a $_Vb_3$ integrin. In a phase I study in adults, Vitaxin given weekly for 6 weeks, the dose was escalated from 0.1 to 4.0 mg/kg/week without observing DLT.[492] Levels sufficient to saturate $a_Vb_3$ integrin were achieved at doses greater than or equal to 1 mg/kg/week, and the half-life at higher doses was in excess of 5 days. A partial response was observed in a patient with leiomyosarcoma, but no objective responses were observed among 15 patients treated in a subsequent phase II trial using a relatively low dose of Vitaxin (0.25 mg/kg/week). [493]

## *EMD 121974 (Cilengitide): A Selective Antagonist of the a $_Vb_3$ Integrin*

EMD 121974, a cyclic pentapeptide containing the RGD sequence, [494] is a potent, selective antagonist of the a $_Vb_3$ integrin. It inhibits *in vivo* angiogenesis in preclinical models of retinal neovascularization and antigen-induced arthritis, [495] and it inhibits tumor growth in xenograft models of melanoma and brain tumors.[496,497] A phase I trial in adults of EMD 121974 given twice weekly has evaluated i.v. doses of 30 to 1,600 mg/m $^2$/infusion.[498] The toxicities observed in this dose range included nausea, anorexia, fatigue, and malaise. They have been mild in nature. Dose-related, linear pharmacokinetics were observed, with a terminal half-life of 3 to 5 hours. Renal clearance of unchanged EMD 121974 accounted for most of the total clearance of unchanged EMD 121974. At the 120 mg/m $^2$/infusion dose, concentrations that optimally inhibited tumor growth in preclinical studies were achieved. [498]

### Pediatric Applications of Angiogenesis Inhibitors

A concern with the development of anti-angiogenic agents in children is the potential for increased susceptibility to toxic effects resulting from angiogenesis inhibition in the developing child. VEGF is necessary during embryonic development, with loss of a single VEGF allele being lethal in the mouse embryo. [499,500] Inactivation of the VEGF gene in embryos leads to impairments in blood vessel development, heart formation, and endochondral bone formation. [499,501] VEGF is also required for

postnatal growth and survival in mice.[502] In newborn mice, high degrees of VEGF inhibition resulted in nearly complete growth arrest and lethality, an effect that diminished with maturation.[502]

Postnatal inhibition of VEGF activity also inhibits endochondral bone formation. [503] The process of endochondral bone formation at the epiphyseal growth plate involves blood vessel invasion of the zone of proliferating chondrocytes. VEGF inhibition blocks the blood vessel invasion and leads to an expanded zone of hypertrophied chondrocytes.[503] Similar effects on the epiphyseal growth plate have been observed for small molecule inhibitors of VEGF and for anti-VEGF monoclonal antibodies.[504,505] The effects of VEGF inhibition on endochondral bone formation are at least partially reversible after cessation of VEGF inhibition. [505]

The basic rationale for studying inhibitors of VEGF signaling and inhibitors of endothelial cell function in pediatric tumors is similar to that for adults. Preclinical studies have demonstrated that antitumor activity can be achieved by inhibiting angiogenesis for a number of pediatric tumors, including neuroblastoma, [6] neurogenic sarcomas,[506,507] Wilms' tumor,[508] rhabdomyosarcoma,[480] and glioblastoma.[509] A phase I trial evaluating SU5416 has been initiated in pediatric patients with primary brain tumors, and clinical evaluations of other agents targeting the VEGF pathway are anticipated. An important strategy to evaluate in using anti-angiogenic agents in children and adults is their combination with chemotherapy and radiation therapy. [510]

One way of combining anti-angiogenic agents with conventional chemotherapy agents is to use the latter at low, frequent doses to take advantage of the anti-angiogenic activities of the agents. [511] In a preclinical neuroblastoma model, the combination of low-dose vinblastine with a monoclonal antibody against the Flk-1/KDR receptor for VEGF resulted in sustained regressions, an effect not seen with either agent used alone. [6] SU5416 is also a potent inhibitor of c-kit, the RTK receptor for stem cell factor, and hence could play a role in the treatment of cancers in which the c-kit receptor is activated. [512]

In terms of studying $a_Vb_3$ integrin antagonists for their anti-angiogenic effect, several pre-clinical studies using pediatric tumor model systems support the potential benefit for this approach in the pediatric setting. For neuroblastoma, expression of a $_Vb_3$ integrin in microvessels of high-risk neuroblastomas is common but occurs much less frequently in low-risk neuroblastomas.[513] In a murine syngeneic neuroblastoma model, EMD 121974 in combination with a tumor-specific antibody–interleukin-2 fusion protein–induced primary tumor regressions, whereas either agent alone induced only growth delays. [5] In an orthotopic nude mouse model for medulloblastoma and glioblastoma, daily treatment with EMD 121974 markedly inhibited tumor growth and caused regressions in some cases. [496] Using the same cell lines, EMD 121974 had no activity when tumor cells were implanted subcutaneously, suggesting that the brain environment was a critical determinant of brain tumor susceptibility to growth inhibition by EMD 121974. As these agents enter clinical trials in newly diagnosed pediatric patients with favorable prognosis, tumor growth delay and arrest will need to be carefully monitored.

## MODIFIERS OF CHROMATIN STRUCTURE: HYPOMETHYLATING AGENTS AND HISTONE DEACETYLASE INHIBITORS

### DNA Methylation

Methylation of cytosine residues in genomic DNA is quite common and usually occurs at cytosine residues adjacent to guanosine (CpG sites). [514] DNA methylation is important in transcriptional repression of imprinted genes and genes subject to X-inactivation, in transcriptional silencing of transposons and viral genes, and in establishing the chromatin structure of some satellite DNA regions. [515] The role of methylation in controlling tissue-specific gene expression is complex, as the promoter regions of these genes often contain CpG islands (regions of DNA with an unusually high frequency of the dinucleotide CpG) that are unmethylated, regardless of the transcriptional status of the genes. However, methylation does appear to play a role in silencing developmentally regulated genes [516] and in some types of tissue-specific gene expression.[517] Methylation results in transcriptional silencing through a linkage between methylation and histone deacetylation, which results in part from methyl-CpG–binding proteins attracting HDAC complexes that then modify chromatin structure, leading to transcription repression. [518,519]

A number of cancers have aberrant methylation patterns, with characteristic patterns of CpG island methylation observed for each type. [520] Abnormal methylation of normally unmethylated CpG islands in the promoter regions of tumor suppressor genes leads to inappropriate silencing of these genes in cancer cells. [521] Tumor suppressor genes and genes involved in apoptosis that are inactivated by abnormal methylation include p16, [521] $p14^{ARF}$,[522,523] caspase-8,[524,525] death-associated protein kinase,[526] and Apaf-1.[527] Treatment of cancer cells with demethylating agents can in some instances lead to reexpression of these genes. [521,523,525,526,527] and [528] Reexpression of components of the apoptotic pathway can increase sensitivity to chemotherapy agents and to other inducers of cell death (e.g., TRAIL). [525,527,528] Thus, demethylating agents may allow reversal of critical steps in oncogenic transformation and reversal of tumor drug resistance.

#### *5-Azacytidine and 5-Aza-2'-Deoxycytidine (Decitabine): Remethylation Inhibitors*

### Background

Agents that inhibit or decrease DNA methylation, for example, 5-azacytidine and 5-aza-2'-deoxycytidine (decitabine), have generated renewed interest because they may allow gene reexpression (Table 11-6). Both 5-azacytidine and decitabine are metabolized to 5-aza-dCTP and subsequently incorporated into newly synthesized DNA, with decitabine being more potent because of its more direct pathway to DNA incorporation. They inhibit DNA methyltransferases by causing an irreversible linkage between enzyme and 5-aza-dC–substituted DNA.[529,530] 5-Azacytidine and decitabine induce differentiation in some cell lines and inhibit cell growth or induce cell death in others.[531,532] and [533] The hypomethylating and differentiating effects of these agents can be observed at concentrations not associated with toxicity and can be isolated from the cytotoxic effects, as the latter are proportional to the quantity of enzyme covalently bound to DNA rather than to the percentage of enzyme inhibition.[533,534] As discussed in the section on HDAC inhibitors, the combination of demethylating agents with HDAC inhibitors can markedly increase reactivation of silenced genes.[535]

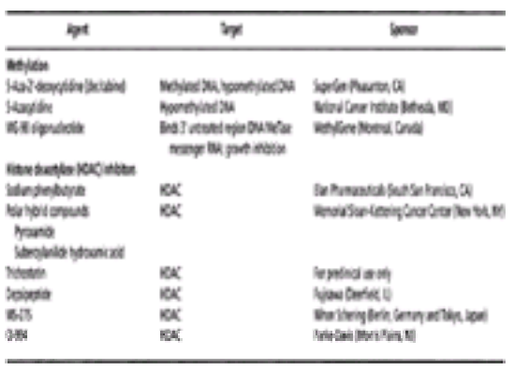


**TABLE 11-6. METHYLATION AND HISTONE DEACETYLASE INHIBITORS**

### Clinical Experience

Although 5-azacytidine and decitabine have been studied in humans for more than 20 years, [532] much of this work was done in the context of developing these agents for their cytotoxic activity rather than for their hypomethylating activity and ability to reactivate silenced genes. Of particular relevance is their use at lower doses for adults with myelodysplastic syndrome, with both 5-azacytidine and decitabine producing hematologic responses in phase II studies. [536,537] and [538] A randomized controlled trial of subcutaneous 5-azacytidine in adults with MDS demonstrated hematologic responses for patients receiving the agent and a trend toward improved survival compared to patients on the observation arm.[539] Both low-dose 5-azacytidine[540,541] and low-dose decitabine[542] have been used in patients with hemoglobinopathies to increase fetal hemoglobin production. Increases in hemoglobin F levels, preceded by DNA hypomethylation, were observed in some patients at doses that had little hematologic toxicity,[540,542] serving as proof of principal demonstrations that noncytotoxic doses of these agents can modify gene expression in a

clinically relevant manner.

### Histone Deacetylase

#### *Histone Deacetylase Inhibitors*

#### Background

Chromosome structure can be modified to alter transcriptional activity by two general mechanisms[543]: (a) histone modification posttranslationally and (b) chromosome remodeling in an energy-dependent fashion.[544] Histone acetyl transferases (HATs) and HDACs regulate the extent of histone acetylation, with transcription being either activated or repressed by recruitment to promoter regions of HATs or HDACs, respectively.[545,546] Given the ubiquitous involvement of HATs and HDACs in controlling gene transcription, it is not surprising that disruption of these chromatin remodeling mechanisms is associated with specific cancers. For example, in acute promyelocytic leukemia (APL), the promyelocytic leukemia (PML)–retinoic acid receptor a (RARa) fusion protein disrupts normal RARa function by enhancing the interaction between PML-RARa and an HDAC co-repressor complex, thereby blocking normal retinoic acid–induced differentiation ( Fig. 11-8).[547] Similarly, in leukemia cells with the t(8;21) translocation and the AML1-ETO fusion protein, there is inappropriate transcriptional repression mediated by the recruitment of HDAC complexes by the fusion protein.[547] Leukemias may also result from translocations resulting in aberrant HAT activation, such as those involving the CREB-binding protein.[547] A more general role for HDACs in cancer is through gene silencing associated with aberrant methylation of CpG islands, a process involving recruitment of HDACs to methylated sequences through the interaction of HDAC complexes with methyl-CpG–binding proteins or with DNMT1.[518,548,549] This interaction between proteins involved in DNA methylation and HDAC complexes provides a conceptual framework for understanding the apparent synergistic gene reexpression observed when hypomethylating agents and HDAC inhibitors are combined.[535,550]

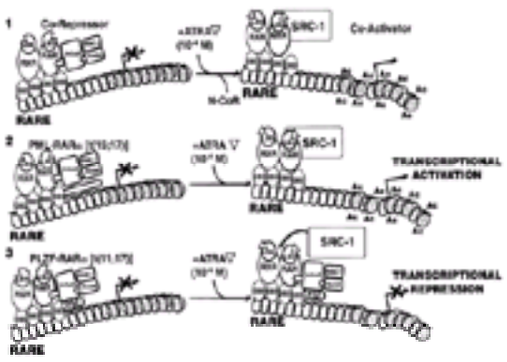



**FIGURE 11-8.** Models for interpretation of the molecular events that are modulated by therapeutic interventions with differentiating agents [all- *trans*-retinoic acid (ATRA)] and histone deacetylase (HDAC) inhibitors in acute promyelocytic leukemia (APL). **1:** The co-repressor complex binds with the retinoic acid receptor a (RARa), promyelocytic leukemia (PML)-RARa, or PLZF-RARa chromosomal DNA translocation site through DNA binding domains (DBD). Binding of the co-repressor complex [RARa, PML-RARa, or PLZF-RARa and nuclear receptor co-repressor (N-CoR), Sin3A, and HDAC1] blocks transcription. Physiologic concentration of ATRA ($10^{-8}$ M) results in release of the co-repressor complex after a conformational change in RARa and attachment of the co-activator, SRC-1, that has histone acetyl transferase activity. After histone acetylation, RARa is transcribed. **2:** In patients with a t(15;17) translocation, therapeutic concentrations of an ATRA ($10^{-6}$M) are necessary for release of the co-repressor complex and subsequent transcription activation after SRC-1–associated acetylation. **3:** In patients with a t(11;17), an ATRA ($10^{-6}$ M) resistant form of APL, therapeutic concentrations of ATRA are not sufficient to cause release of the co-repressor complex (N-CoR or Sin3A and HDAC1) that is bound to POZ. POZ binding makes the ligand binding domain (LBD) insensitive to ATRA and apparently blocks SRC-1 co-activator binding, thus inhibiting histone acetylation, leading directly to transcription repression. The role HDAC inhibitors play in release from transcription repression needs to be determined. Ac, histone acetylation; ARC-1, co-activator with histone acetyltransferase activity; Rare, retinoic acid response element; RXR, retinoic acid receptor. (Adapted with permission from Collins SJ. Acute promyelocytic leukemia: relieving repression induces remission. Blood 1998;91:2631–2633 and Guidez F. Ivin S, Zhu et al. Reduced retinoic acid—sensitivities of nuclear receptor corepressor binding to PML- and PLZF-RARa underlie molecular pathogenesis and treatment of acute promyelocytic leukemia. Blood 1998;91:2634–2641.)

Several classes of HDAC inhibitors have been studied, including hydroxamic acid–based hybrid polar compounds, for example, suberoylanilide hydroxamic acid and pyroxamide,[551] synthetic benzamide derivatives (e.g., MS-27-275),[552] short-chain fatty acid derivatives (e.g., butyric acid, phenylbutyrate, and phenylacetate),[553,554] and cyclic peptides (e.g., depsipeptide)[555] (Table 11-6). Inhibitors of histone deacetylation have multiple effects *in vitro*, including inducing growth arrest, differentiation, and apoptotic cell death.[556,557 and 558] *In vivo* testing of the HDAC inhibitors has demonstrated marked inhibition of tumor growth in a number of tumor models (e.g., prostate, colorectal, and ovarian tumor lines).[552,559]

#### Clinical Experience

A butyric acid derivative, phenylbutyrate, was the first HDAC inhibitor to enter clinical evaluation, but it has the disadvantage of requiring millimolar concentrations for HDAC inhibition. Although the biologic effects of phenylbutyrate are not restricted to inhibition of histone deacetylation,[560] the clinical experience with this agent does suggest the potential anticancer utility of targeting HDACs to alter gene expression. Phenylbutyrate induced fetal hemoglobin production in patients with hemoglobinopathies,[561,562] and phenylbutyrate was able to overcome resistance to all-*trans*-retinoic acid (ATRA) in a patient with APL, an effect presumably related to phenylbutyrate inhibition of the HDAC complex recruited by PML-RARa.[563] Current clinical research focuses on the more potent HDAC inhibitors that show activity at nanomolar or micromolar concentrations.[557] Among the more potent HDAC inhibitors that are in early clinical development are depsipeptide,[564] MS-27-275,[560] and suberoylanilide hydroxamic acid.[557]

#### Pediatric Applications

Demethylating agents may have a particularly important role for pediatric tumors such as neuroblastoma and medulloblastoma, in which silencing of the caspase-8 gene occurs and is associated with resistance to apoptosis.[524,525,565] Reexpression of caspase-8 by decitabine leads to increased sensitivity to TRAIL-induced apoptosis[525,565] and increased sensitivity to chemotherapy,[527] suggesting the strategy of using demethylating agents in combination with apoptosis-inducing agents to increase the efficacy of the latter. Transcriptional silencing by methylation occurs in other pediatric cancers, for example, p15 and p16 inactivation in T-cell ALL,[566] and demethylating agents may warrant evaluation for these diagnoses.

HDAC inhibitors may be particularly relevant for pediatric leukemias caused by fusion proteins that recruit HDAC complexes to promoter regions, including APL with RARa fusion proteins,[567] AML with AML1-ETO fusion proteins,[568] and ALL with TEL-AML1 fusion proteins.[569] HDAC inhibitors may also be active against pediatric solid tumors, as illustrated by the ability of a hybrid polar HDAC inhibitor to inhibit cell growth and induce apoptosis in neuroblastoma cell lines[556] and to enhance retinoid sensitivity in neuroblastoma cell lines.[570] As seen in adults, the enhanced ability to reexpress silenced genes through the combined use of a demethylating agent and a HDAC inhibitor[535,550] is a promising approach for those tumors in which gene silencing plays a role in cancer development or in resistance to therapy.

## REACTIVATION OR PERSISTENT ACTIVATION OF TELOMERASE

The primary role of the telomere is to protect the chromosome from recombination, fusion, or recognition as damaged DNA.[571,572] Telomerase is an RNA/protein complex. With every cell division, the chromosome becomes fused, lost, or unstable in normal cells compared to cancer cells as the telomere is shortened. Telomere shortening leads to lack of cell division and proliferation and eventually apoptosis.[573] Cellular senescence usually occurs because the molecular cap, the telomere, wears away after multiple replications. Replication of telomeric DNA is a cellular survival mechanism. The cancer cell appears to be able to repair eroded telomeres and, therefore, its survival is enhanced. Chromosomal degradation and DNA ligation by DNA repair enzymes is prevented by the presence of the telomere.

Maintaining the length of the telomere is one of the factors that allows for unrestricted proliferation in tumor cells. [574]

Alteration in cellular life span is circumvented in cancer, germ cells, and embryonal cells by the reactivation or persistent activation of telomerase. [575] Telomerase activity is lost after birth in human somatic cells.[576] Poor-prognosis neuroblastoma, melanoma, breast, esophageal, and pancreatic cancer exhibit telomerase activity.[577,578,579,580,581 and 582] Cellular immortalization occurs with telomerase activity, and Rb and p16 $^{ink4a}$ inactivation.[583]

Telomerase copies DNA using an RNA template repeat, TTAGGG, that may be up to 1,000 bases long.[575] These template repeats bind to TRF1,[584] and TRF2,[585] telomere repeat-binding factors. Approximately 80% to 90% of cancer cells contain telomerase, specifically the hTERT catalytic subunit. [575] Telomerase also has telomerase reverse transcriptase activity that catalyzes TTAGGG synthesis. Diminished telomere length, arrested cell growth, senescence, and apoptosis are inhibited in a competitive fashion by an inactive telomerase reverse transcriptase. [572]

### Antisense Oligonucleotides or Peptide Nucleic Acid Oligomers

Telomerase inhibitors include antisense oligonucleotides or peptide nucleic acid oligomers targeted to hTR or hTERT, and elongation of guanine-quadruplex structures. Inhibitors of the telomerase complex include 2'-O-methylated RNA and peptide nucleic acid oligomers that bind or compete with the consensus sequences.[586] Telomerase activity has been inhibited by a hammerhead ribozyme with the catalytic domain flanked by sequences complementary to RNA telomerase and by 2'-O-methyl-modified ribozyme.[586] The 5' peptide nucleic acid ends conjugated with cationic peptides have $IC_{50}$ values in the 0.14 µM range.[586,587] Antisense approaches are limited by ineffective membrane transport of these agents.

### Amidoanthraquinones

Three isothiazolones inhibit telomerase with $IC_{50}$ values of 130 to 140 nM. These agents bind near the active site or modify cysteine residues. [588] The amidoanthraquinones inhibit telomerase by binding the primer strand of the telomere and preventing the formation of the guanine-quadruplex structures during elongation.[589,590,591 and 592] Many other agents inhibit telomerase, including tetrapyridyl-substituted cationic porphyrins, [593] peryline derivatives,[594,595] acridines,[596,597] fluorenones, and regioisomers of bis-substituted amido-anthraquinones. [590,591 and 592]

### Alkaloid Berberines

Using the COMPARE analysis program from the Developmental Therapeutics Program of the National Cancer Institute, [598] other telomerase small molecule inhibitors were identified. Alkaloid berberines and mitochondria-accumulating agents were tested for their ability to inhibit telomerase. A rhodacyanine derivative, MKT077, [598] was chosen as a lead compound. Using MKT077 as the lead for the COMPARE analysis, a highly potent compound, FJ5002, with an $IC_{50}$ of approximately 2 µM was identified; this agent has not been tested in humans yet. FJ5002 demonstrates the characteristics of specific telomerase inhibitors: telomere erosion, chromosomal abnormalities, and senescence.

#### *MKT077*

MKT077 was administered as a 30-minute infusion weekly for 4 of 6 weeks in a phase I clinical trial. [593] Thirteen patients received treatment, using doses ranging from 42 to 126 mg/m$^2$/week. Because nephrotoxicity was observed in rodent models, forced diuresis with mannitol was started at the 84 mg/m$^2$ dose. The adverse events observed on study included emesis, edema, cumulative renal magnesium wasting, and renal dysfunction. A three-compartment model best describes the pharmacokinetic data for the first two dose levels. The elimination half-life a and g ($t_{1/2}$ a and the $t_{1/2}$ g) were 1.0 to 2.5 minutes and 25 to 30 hours, respectively. The mean AUC was 1,000 mg·h/mL at the 42 mg/m$^2$/week dose level and 1,850 mg·h/mL at the 56 mg/m$^2$/week dose level. The $C_{max}$ was 1,140 and 2,870 mg/mL for the two dose levels tested thus far. The clearance of MKT077 was 39.2 ± 11.4 L/hour/m$^2$ and was independent of the dose. This study confirms a tolerable dose and schedule that achieves a plasma concentration similar to the $IC_{50}$ concentrations required to sensitize human tumor cell lines. The primary *in vivo* mechanism of action of MKT077 may not be telomerase inhibition, but the alkaloid berberines have provided leads for the identification of more active inhibitors of telomerase in preclinical screens.[593,598]

## DEVELOPMENT OF MOLECULAR-TARGETED AGENTS FOR CHILDHOOD CANCERS

Four key steps in identifying and prioritizing molecular-targeted agents for pediatric indications are (a) identifying critical survival and apoptotic pathways in childhood cancers, (b) identifying agents that modulate these signaling pathways, (c) defining appropriate doses and schedules for these agents in children, and (d) incorporating these agents into the multi-agent, multimodality regimens used to treat children with cancer.

### Identifying Critical Survival and Apoptotic Pathways in Childhood Cancers

The first step in prioritizing a molecular-targeted therapy for evaluation in children is identifying the alterations in cell death signaling that specific cancers have acquired (either before or after treatment) and identifying the cell survival signaling pathways that are activated. Overexpression of bcl-2 and other anti-apoptotic members of the bcl-2 family (e.g., Bcl-$X_L$) may play a role in cell survival and treatment resistance for AML [28,30,31] and neuroblastoma.[25] Signaling through the IGF-1 receptor appears to be an important proliferation and survival pathway for rhabdomyosarcoma, [599,600] neuroblastoma,[601,602 and 603] Wilms' tumor,[604,605] desmoplastic small round cell tumor,[130,606,607] and Ewing's sarcoma.[608] These examples of potential targets may be exploitable for therapeutic gain in the treatment of childhood cancers.

### Identifying Agents That Modulate Critical Signaling Pathways

A second step in applying molecular-targeted therapies for children with cancer is to identify agents that modulate key steps in cell survival and cell death pathways. For some agents there may be relatively high specificity for a specific pathway activated in cancer cells. The prototypical example of a molecular-targeted therapy is STI571, which by inhibiting Bcr-Abl tyrosine kinase associated with CML and Ph$^+$ ALL, induces cells expressing this fusion protein to undergo apoptosis. [110,113,609] Clinical trials of single-agent STI571 demonstrated the ability of the agent to induce complete remissions in both CML and Ph$^+$ ALL,[125,126] serving as a proof of principle that specific inhibitors of critical signaling pathways can be effective anticancer agents. For other molecular-targeted agents, the linkage between the effect of an agent on its target and cell survival/cell death pathways is less direct. For example, HDAC inhibitors induce apoptosis in a number of different cell types *in vitro* (including AML cells and neuroblastoma cells). [556,558] Their activity is probably the result of the induction of the transcription of genes (as yet unidentified), leading to eventual apoptosis. Proteasome inhibitors induce apoptosis in a number of tumor cell lines *in vitro*, including Ewing's sarcoma,[304] chronic lymphocytic leukemia,[610] Jurkat T cells,[611] and AML.[612] Multiple changes occur in cells treated with proteasome inhibitors that may affect cell growth and survival, including stabilization of p53,[613] inhibition of NF-kB activity,[288,610,614] accumulation of the cdk inhibitors p21 and p27,[611] and accumulation of the pro-apoptotic protein Bax.[615]

### Defining Dose and Schedule for Molecular-Targeted Agents

A third step in the development of molecular-targeted therapies for pediatric cancers is defining an appropriate dose to take into phase II and subsequent evaluations. Given the limited number of children with cancer eligible for early phase trials, defining optimal dosing and scheduling to modulate an agent's target will most often be done in adults. Dose-finding studies in adults have traditionally escalated to DLT. For some targeted agents, however, there may be a large difference between the doses that cause modulation of the agent's target and the doses that cause significant toxicity in patients, and for these agents, a biologically active dose may be determined rather than an MTD.

In the ideal setting of an agent that is specific for a target critical for the survival of the cancer cells, decisions about dosing can be based primarily on antitumor activity. The application of STI571 for Ph$^+$ leukemias is an example in which high levels of antileukemia activity were observed in phase I trials, and the selection of dose could largely be based on response rate.[112,125,126] In the absence of antitumor activity, investigators must develop direct measures of target effect on tumor cells or target effect on normal tissue (e.g., peripheral blood mononuclear cells, buccal cells, skin). Potentially useful biologic correlative end points for molecular-targeted agents include binding or receptor saturation, expression at the RNA or protein levels, receptor activation or phosphorylation in tumor tissue, or measurement of

circulating proteins. Generally such tissue measurements should be correlated with pharmacokinetic studies. Again, biologic activity documented by functional imaging needs to be developed and validated for digital-contrast enhanced MRI; positron emission tomography using isotopes including oxygen-15, carbon-11, and fluorine-18; Doppler ultrasound; helical computed tomography; and three-dimensional radiolabeled imaging (single-photon emission computed tomography). Imaging modalities may be especially applicable for evaluating the biologic effects of anti-angiogenic agents. [616]

The decision-making algorithm for determining optimal dosing based on biologic activity as measured in tumor tissue after treatment is complex and may be based on assumptions of unknown validity. For example, the degree of target inhibition required to obtain the maximum antitumor effect and the duration of target inhibition required for optimal antitumor activity may be unknown. The effect of interpatient tumor heterogeneity may be difficult to characterize in limited numbers of patients. The impact of intrapatient tumor heterogeneity and the possibility of introducing sampling error from serial biopsies of a heterogeneous tumor must also be considered. Furthermore, the putative target may not be the only biologically relevant target for the drug, and higher doses might be required to inhibit other unrecognized targets that contribute to antitumor activity. Given all of these uncertainties, in many cases it will be deemed beneficial to escalate to doses with clear biologic effects (i.e., responses or toxicities) or to provide a substantial cushion above the minimal dose modulating the agent's target.

### Incorporation of Molecular-Targeted Agents into Multi-Agent, Multimodality Regimens

A final general consideration in the development of molecular-targeted therapies is how they should be incorporated into regimens with standard therapy. For many targeted agents, rationale for combining targeted new agents with conventional agents comes from preclinical data demonstrating enhanced antitumor activity for the combinations. Of particular interest for use in combination therapy are the targeted agents that in some way tilt the balance of survival and death signals in favor of apoptosis. For example, STI571 not only induces apoptosis in Bcr-Abl expressing cells but also potentiates the cytotoxic activity of conventional cytotoxic agents such as Ara-C and doxorubicin.[119] UCN-01, a staurosporine analog that inhibits the DNA damage checkpoint kinase hChk1, [200,201] markedly potentiates the cytotoxic activity of a number of chemotherapy agents,[617] including topoisomerase I inhibitors, [618] Ara-C,[619] and cisplatin.[620] Similarly, proteasome inhibitors potentiate the cytotoxic activity of cisplatin,[621] doxorubicin and etoposide,[283] and ionizing radiation.[285] Other examples illustrating the potential benefit for combining molecular-targeted agents with cytotoxic agents are described in the discussions of individual agents.

The development of combinations of standard agents with chemosensitizing molecular-targeted agents is complicated by the difficulty in obtaining preliminary evidence for benefit for the addition of the targeted agent. The ideal solution (in terms of obtaining a reliable answer) is to conduct a randomized trial of the combination with and without the targeted agent, but this is impractical to do for all of the possible combination regimens of interest. An alternative method that can be used to screen new combinations that include a molecular-targeted agent is comparison of outcome (e.g., response rate and event-free survival) for patients receiving the combination with the molecular-targeted agent to outcome for historical controls. Alternative solutions to historical controls are reviewed elsewhere. [622] This requires a well-characterized control population and evidence for comparability between the historical control group and the experimental group.

### Pediatric Molecular Markers

Researchers have sought prognostic and predictive markers of tumor behavior to guide both the design of clinical trials and the selection of therapy for individual patients. Prognostic markers indicate the likelihood of recurrence or survival regardless of treatment. Only recently has the research effort into prognostic and predictive markers for childhood cancers begun to intersect the research effort into targets of therapy. Marker studies generally have not been pursued with the rigor applied to clinical trials of therapeutic agents. The literature is replete with reports of research on markers in small groups of patients. Rarely is a comparable study performed to replicate results, and even more rarely is a prospective, randomized clinical trial designed with sufficient statistical power to test the utility of a marker. There is enormous scope for future research into both the basic cancer biology of childhood cancers and into the clinical application of current and future knowledge.

## CONCLUSIONS

The development of molecular-targeted therapies for childhood cancers will need to address the therapeutic window, as cancer cells may be preferentially dependent on the survival pathways or preferentially susceptible to the cell death pathways targeted by the agent when compared to normal cells. Many of the molecular-targeted agents modulate protein activity in normal and malignant tissues. Molecular-targeted agent specificity for tumor cells may not be dramatically different from currently used agents such as vincristine, topotecan, and etoposide. These agents target ubiquitous proteins, tubulin, and topoisomerase I and II, respectively. Other molecular-targeted agents modulate the activity of proteins that have more limited effects and limited distribution. Even though the targets are expressed on many normal cells, agents that inhibit RTKs may have a more limited effect on many normal tissues. Many molecular-targeted agents may be safely administered at doses that modulate their target. Alterations in the balance of cell death and survival in normal, nonhematopoietic tissue may lead to events that produce significant long-term effects.

The multiple mechanisms by which cancer cells promote their own survival and avoid cell death underscore the opportunities available for therapeutic intervention; the determination of which pathways are central to treatment resistance for specific cancer types should be given careful consideration as pediatric development of molecularly targeted therapies are undertaken. The understanding of essential survival and cell death pathways continues to expand; as an increasing number of agents that target specific pathways enter the clinic, molecular-targeted therapies may lead to more effective treatments for children with cancer.

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# 12

# GENERAL PRINCIPLES OF SURGERY

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## INTRODUCTION

Surgery remains a critical component in the multimodal therapy of childhood cancer. Accurate staging and successful resection of tumors is of vital importance as we strive to minimize the morbidity of treatment as survival rates improve. Decisions regarding biopsy and resection are most accurately made by surgeons who are trained to address these specific issues. This chapter presents an introduction to the significant considerations for surgical patients, including their metabolic response, nutritional support, and vascular access. Critical issues regarding anesthetic management, staging, biopsy, and preoperative adjuvant therapy are also discussed. A brief summary of some of the specific surgical issues for each solid tumor concludes the chapter.

## GENERAL PRINCIPLES

### Metabolic Response to Malignancy

Weight loss is frequently seen in children with malignancy. This form of malnutrition, termed *anorexia-cachexia syndrome*, is associated with higher early cancer relapse rates, decreased tolerance to chemotherapy, and a poorer prognosis.[1] Although reduced food intake is an important component of the syndrome, cancer patients, unlike starved individuals, have elevated protein turnover and lose the ability to conserve skeletal muscle mass.[2] Although these changes are seen in most malignancies, the extent of the response may be more pronounced in those with high cell turnover. Other notable metabolic changes with malignancy include increased rates of gluconeogenesis and lipolysis. There is also a failure to downregulate energy expenditure in response to reduced nutrient intake, although actual increases in resting energy expenditure are only evident in patients with large tumor burdens.[3] Surgical stress or infection tends to further exacerbate the net protein catabolism found in these patients.[4]

The pathogenesis of childhood cancer cachexia is complex and incompletely understood. Elaboration of host cytokines in response to the tumor, such as tumor necrosis factor-alpha, interleukin-1, interleukin-6, and interferon-gamma, promote catabolism, which in certain instances is further enhanced by specific tumor generated mediators.[5] The cytokine-independent ubiquitin-proteasome pathway has also been linked to muscle protein loss in cancer patients.[6,7] Compounding this catabolic process is a reduction in enteral intake and, on occasion, malabsorption of nutrients that may be caused by the tumor itself, antineoplastic therapy, or a combination of both.

### Nutritional Support

In the absence of efficacious pharmacologic interventions to combat the anorexia-cachexia syndrome, nutritional support remains the primary therapy. Children are particularly susceptible to the deleterious effects of cancer-induced catabolism due to their relatively reduced stores and high baseline requirements. The concern that the provision of nutrition may stimulate tumor growth in children is not supported by clinical data. There is also no evidence that the administration of macronutrients and micronutrients in excess of those recommended for other ill patients is required.

The use of preoperative nutritional support in children with malignancy has not been systematically studied; however, extrapolation from the adult literature would indicate that preoperative nutritional repletion should be considered only in those patients with preexisting malnutrition. Postoperatively, any child who is anticipated to have inadequate nutritional intake for more than 3 days should be considered as a candidate for nutritional support.

Enteral nutritional support is more physiologic, has lower complication rates and is less costly than parenteral nutrition. The advent of pliable, narrow bore, nasogastric feeding tubes and percutaneously placed endoscopically guided gastrostomy tubes has facilitated this option in children with cancer. The latter tubes have been shown to be safe in pediatric cancer patients and should be considered if protracted nutritional support is anticipated.[8] The conversion of gastrostomy tubes to gastrojejunal tubes is occasionally necessary to limit gastroesophageal reflux. Parenteral nutritional support is recommended for those children who are not suitable for enteral nutrition and adequate amounts of protein, glucose, lipid, trace minerals, and vitamins, commensurate with a metabolic stress state, should be administered.[4]

### Vascular Access

Vascular access procedures in oncology patients continue to be a dominant and critically important component of the total care plan.[9] In a child who must experience 6 to 24 months of primary or adjunctive chemotherapy, radiotherapy, and operative procedures, predictable as well as long-standing central venous access is important for obtaining diagnostic blood samples as well as for administering fluid and electrolytes, blood products, parenteral nutrient solutions, antibiotics, and other medications, as well as the anticancer therapies themselves.

Venous access selection for an oncology patient must take into consideration duration of projected need, frequency and duration of projected access into the vascular system, availability of patent veins for puncture, coagulation status of the patient, as well as experience of the surgeon. Typically, central venous catheters can be defined as external or implantable (subcutaneous infusion port). Furthermore, external catheters may be inserted directly into a central vein—subclavian, internal jugular, saphenofemoral—by percutaneous or cutdown techniques or a new technique of peripheral insertion of a central venous catheter that has also evolved.[10]

Totally implantable ports offer the advantage of no external catheter, thus improving body image and removing a putative limitation of physical activity. However, any potential for enhanced protection from infection when the device has been totally internal has been controversial. [11,12] Another important consideration in pediatric vascular access is the need for general anesthesia for central catheter placement, selected tunneled catheter removal, and for the removal of all implanted subcutaneous infusion ports.

Peripherally inserted central lines are typically placed at the bedside using topical and injectable analgesia, sterile technique, and chest radiograph to confirm catheter position. Most commonly, antecubital veins are the first choice for access, and the catheter exit site is maintained with frequent dressing changes and topical antibiotics with coverage by a transparent adhesive bandage, which permits visualization of the catheter at the site of entry. The major limitation of this technique is a greater likelihood of catheter malposition, mechanical catheter damage, inadvertent dislodgement, and phlebitic change in the smaller peripheral access vein. [10]

Central venous catheters are most commonly placed using the percutaneous Seldinger technique accessing the subclavian vein via the infraclavicular approach. A cuffed catheter is exited through a remote tunnel, the cuff and the tunnel both being mechanical factors that may limit the progression of bacteria along the catheter. Alternate access sites for catheter placement include the internal jugular, external jugular, and femoral veins, but ease of directing the catheter tip into the vena cava–right atrial junction along with achieving tunneling of the catheter is most effectively achieved with subclavian vein access. In contrast to venous selection for percutaneous access, if an open cutdown technique is instead selected to minimize placement complications, then the external or internal jugular veins and the saphenofemoral junction are preferred access routes.

Placement of a totally implantable device is optimal for the adolescent receiving intermittent cyclic chemotherapy. Again, venous access is obtained by either a percutaneous or cutdown technique, and the catheter is tunneled to the port's subcutaneous pocket placed either superior or inferior to an incision on the chest wall. The diaphragm of the port is placed in a palpable position, which allows ease of puncture by a metallic Huber needle, and the body of the port is secured by a series of sutures to the underlying muscle fascia.

Complications of central venous access are mechanical (related to the venous access procedure or the catheter itself), infectious, or infusion related. With percutaneous subclavian vein access, the greatest patient risk is pneumothorax, hemorrhage from either arterial puncture or vein laceration, pericardial tamponade secondary to atrial or ventricular penetration, or needle puncture–related neurologic (vagus, phrenic, or sympathetic trunk) or thoracic duct injury. Percutaneous access to the jugular vein carries similar risks, carotid artery penetration being a not uncommon event. Of concern is the very real risk of a life-endangering event from the vascular access procedure alone, a risk that approximates 1 such event per 1,000 access procedures. [11]

Infectious complications of pediatric central venous access remain most ommon.[12] Definition of such catheter-related bloodstream infections as opposed to local exit-site, tunnel, or pocket infections is important to analyze. The local site infections typically account for $^1/_5$ to $^1/_2$ of all central access infections and present with local signs and symptoms, including fever, erythema, tenderness, and drainage. In contrast, bloodstream infections are diagnosed when a presumptive diagnosis of bacteremia or sepsis is made and there is congruence of the cultured organism from a catheter segment and a venous blood culture. [13] However, as transcatheter antibiotics are typically initially administered in an effort to salvage the infected line, the presumption of a catheter-related infection is usually made on clinical suspicion with an inability to culture the catheter itself. The pediatric age group has remained a high-risk group for such infections. The microbiology of such infections are predictable, coagulase-negative staphylococci almost uniformly being the most common offending organism. [12,13] In all series, but especially for the immunocompromised population of oncology patients, the spectrum of causal organisms includes other gram-positive, many gram-negative, and, not infrequently, fungal species. Factors that may protect patients from infection historically included the aseptic technique of the operating room, the tunnel, and the catheter cuff. To these have been added antibacterial substances bonded to catheters in which minocycline and rifampin supply an added advantage. [14]

Risk factors for the pediatric patient that influence central catheter infections have been carefully analyzed. [12] Early infection occurred in 12% of children (53 of 437), and factors that adversely influenced this rate included moderate (absolute neutrophil count <1,000) or severe (absolute neutrophil count <500) neutropenia and failure to use perioperative prophylactic systemic antibiotics. Type of catheter and site of placement did not influence infectious risk. In this series, as in many others, transcatheter antibiotics as an initial therapy in presumed central line infection was effective in treating that infection and preserving the catheter in 70% of the patients.[12] Such transcatheter antiinfective agents worked less well for the child with fungemia. Furthermore, because of the demonstrated relationship between thrombosis and infection, thrombolytic agents, anticoagulants, as well as antibiotics, have been used to treat the infected catheter. [15]

## ANESTHESIA

### Principles of Perioperative and Anesthetic Management

Although the perioperative and anesthetic management of children with malignancy is generally similar to other ill pediatric patients, the following aspects warrant more detailed consideration: coagulation and transfusion, tumors that result in anterior mediastinal masses, and pheochromocytomas. In patients already treated with chemotherapy, a careful assessment of cardiac, pulmonary, renal, and electrolyte status is particularly warranted. Patients who have received cardiotoxic chemotherapy regimens, such as those including adriamycin, often require echocardiography to quantitatively document cardiac function before surgery.

#### *Coagulation and Transfusion*

Thrombocytopenia is the most commonly encountered clinical coagulation problem in children with malignancy requiring surgery. Platelet counts of 150,000 per $mm^3$ are the lower limit of the normal range in children, whereas normal neonates may have platelet counts as low as 80,000 per $mm^3$. Patients with platelet counts greater than 10,000 per $mm^3$ have a low risk of spontaneous bleeding if they are without evidence of other medical complications. [16] If surgery is contemplated, however, a platelet count of 50,000 per $mm^3$ is usually sought before incision. This guideline is empirically based and has not been formally studied; hence, lower levels may be acceptable to the operating surgeon in specific clinical contexts. In children, the transfusion of 0.1 units of platelet concentrate for each kilogram of body weight may be anticipated to raise the platelet count 40,000 per $mm^3$.

No specific hemoglobin concentration or hematocrit level mandates blood transfusion in an oncologic surgical patient. Children with hemoglobin levels of 6 g per dL are often free of adverse physiologic sequelae. The specific clinical scenario governs the need for transfusion, as rapid blood loss, infection, pulmonary dysfunction, cardiomyopathy, and central nervous system compromise may all mandate early transfusion. Immunodeficient patients should receive irradiated blood products and, if the patients are serologically cytomegalovirus-negative, they should receive cytomegalovirus-negative or leukocyte-poor blood products. Packed red blood cells are the usual blood product used for both acute blood loss and chronic anemia. Although with massive hemorrhage whole blood is a suitable choice, it is rarely available.

Elevations in prothrombin time (PT) and partial thromboplastin time (PTT) are acquired defects in most surgical patients. Vitamin K deficiency manifests with a marked prolongation of the PT and PTT and normal platelet and fibrinogen levels. In older children, this may be due to malabsorption, dietary deficiency, or drug antagonism. The subcutaneous administration of vitamin K results in improvement within several hours and correction within a day. For immediate correction, fresh frozen plasma is required. If the platelet count and fibrinogen are also low, disseminated intravascular coagulation must be considered, particularly in patients with sepsis or leukemia. Peripheral blood smears, platelet count, thrombin time, fibrin degradation products, fibrin monomers, and D-dimer levels are all useful in confirming the diagnosis. Treatment of disseminated intravascular coagulation consists of correction of the underlying disease and supportive care. If hemorrhagic manifestations are present without major thrombosis, fresh frozen plasma may be indicated. Liver disease with decreased synthetic capacity may also result in an elevated PT and PTT refractory to vitamin K.

#### *Evaluation of Anesthetic Risk of an Anterior Mediastinal Mass*

Respiratory collapse on induction of general anesthesia is a well-recognized complication of an anterior mediastinal mass ( Fig. 12-1). Identification of which patients are at significant risk remains a major challenge. Orthopnea, respiratory stridor, and wheezing are all ominous signs of significant major airway obstruction ( Fig. 12-2). Several patients with no respiratory symptoms, however, have been reported to develop major respiratory complications. [17]

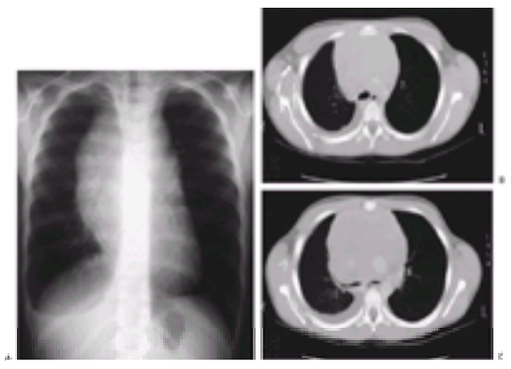

**FIGURE 12-1. A:** An 11-year-old girl presented with a several week history of cough and dyspnea on exertion and a 3-day history of puffy eyes and orthopnea. The chest radiograph reveals a large anterior mediastinal mass and a right pleural effusion. **B:** Computed tomogram showed a tracheal area that was 82% of predicted. **C:** However, scans obtained lower in the chest revealed significant narrowing of both bronchi. This finding probably explains the marked reduction in her peak expiratory flow rate, which was 42% of predicted while sitting and only 24% of predicted in a supine position. The diagnosis of lymphoblastic lymphoma was established by aspiration of her pleural effusion.

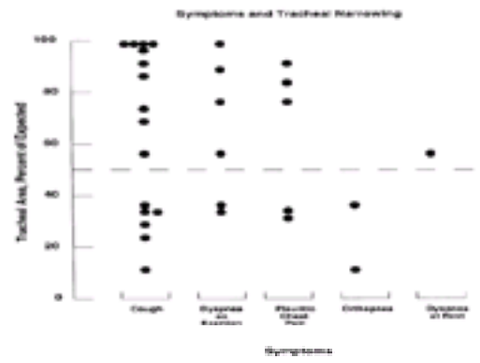

**FIGURE 12-2.** Correlation of symptoms and tracheal areas from a cohort of 42 children and adolescents with an anterior mediastinal mass. (From Shamberger RC, Holzman RS, Griscom NT, et al. CT quantitation of tracheal cross sectional area as a guide to the surgical and anesthetic management of children with anterior mediastinal mass. J Pediatr Surg 1991;26:138–142, by permission of the publisher, W.B. Saunders, Co.)

Computed tomography (CT) is useful in defining the cross-sectional area of the trachea, and a reproducible technique for defining the tracheal area has been published.[18,19 and 20] The cross-sectional area is measured from the CT slices showing the trachea at its narrowest dimension. The percentage of narrowing is then calculated by comparing the measured area with established standard values. [21]

The use of pulmonary function tests in evaluating children with an anterior mediastinal mass is also important. The predominant distortion of the flow loop for intrathoracic obstruction is a marked reduction in the maximum expiratory flow rate. For extrathoracic obstruction, it is a reduction of the maximum inspiratory flow rate. A fixed lesion usually produces an equal reduction of the inspiratory and expiratory peak flows. The high-flow portion of the forced expiratory loop near total lung capacity, best represented by the peak expiratory flow rate (PEFR), is the first to be distorted.

The PEFR can be easily obtained in patients using a hand-held device. A prospective evaluation of pulmonary function and tracheal area in 31 children with mediastinal masses was performed before 34 surgical procedures.[22] The tracheal area (as a percent of the predicted area) was determined by CT. In this study, criteria for administration of local anesthesia were either a tracheal area of less than 50% of predicted or a PEFR of less than 50% of predicted. All children administered anesthetics following these guidelines had an uneventful intraoperative course ( Fig. 12-3). The study did not prove that children with a PEFR of less than 50% of predicted would experience respiratory collapse with general anesthesia, because all children who met this criterion were excluded from receiving general anesthesia. It did confirm, however, that general anesthesia could be safely used in children who met the minimum criteria of PEFR and tracheal area of greater than 50% of predicted. As treatment began and the masses shrank, the pulmonary function tests and total lung capacity improved.

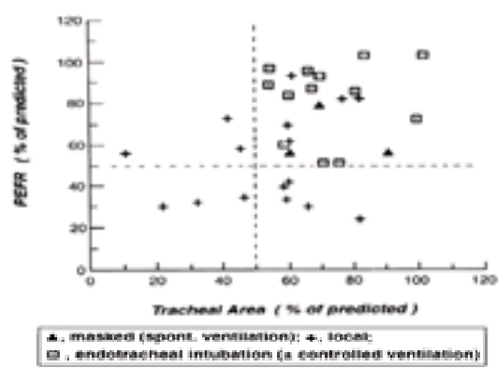


**FIGURE 12-3.** This graph shows the correlation between peak expiratory flow rate (PEFR) and tracheal area in a cohort of 31 children prospectively evaluated with an anterior mediastinal mass. Children with PEFR and tracheal area less than 50% of predicted, to the left of and below the dashed lines, all received local anesthetic and did well. Those children with PEFR and tracheal area greater than 50% of predicted received predominantly general anesthesia and did well. The five children with tracheal area greater than 50% of predicted, in the lower right box, might have been considered for general anesthesia if tracheal area was the only parameter considered to assess risk. Although the study could not demonstrate that these children would have had anesthetic problems, it did confirm that these parameters (greater than 50% of PEFR and tracheal area) were safe for the administration of general anesthesia. (From Shamberger RC, Holzman RS, Griscom NT, et al. Prospective evaluation by computed tomography and pulmonary function tests of children with mediastinal masses. Surgery 1995;118:468–471, by permission of the publisher Mosby-Year Book, Inc.).

In patients with Hodgkin's and non-Hodgkin's lymphoma (NHL) who had their pulmonary status evaluated, those with NHL appeared to be more impaired. [23] Respiratory symptoms were more common in the children with NHL than in those with Hodgkin's disease. The pulmonary function tests were also worse in the NHL cohort, and these tumors were shown to account for a disproportionate number of the larger masses. A correlation between the size of the mass and the impairment in pulmonary function was also noted.

Children will inevitably be encountered with significant respiratory compromise that require biopsy, but will not tolerate a general anesthetic. The presence of extrathoracic tissue for biopsy, particularly cervical lymphadenopathy, should be sought in all cases, but in some children it will be entirely lacking. It was demonstrated in the late 1990s that children with lymphoblastic lymphoma have a significantly higher incidence of an associated pleural effusion than in children with Hodgkin's disease.[24] This study has also demonstrated that aspiration of the effusion was helpful in providing a diagnosis of lymphoblastic lymphoma in all three children in whom it was performed. The diagnosis of T-cell lymphoblastic lymphoma has also been established by immunocytochemical studies of pleural fluid in two childhood cases presenting with strictly intrathoracic lesions. [25] Cytogenetic evaluation of cells obtained from pleural fluid can be helpful and diagnostic as well. The (t 8; 14) (q 24; q11) translocation is particularly associated with T-cell lymphoblastic leukemia and the related T-cell lymphoblastic lymphomas, and its identification will

facilitate their diagnosis.[26] Immunophenotyping of the cells obtained from pleural fluid can also show a predominance of T- versus B-cell markers, supporting the diagnosis of lymphoblastic lymphoma.

In children with significant respiratory compromise and no pleural effusion for aspiration and no extrathoracic disease, then either an intrathoracic biopsy under local, a percutaneous radiographically guided needle biopsy, or preliminary treatment with radiotherapy with shielding of an area for future biopsy must be performed. Needle biopsies are often successful in establishing a diagnosis of NHL, but are less helpful in cases of Hodgkin's disease. At our institution, local biopsies using a transthoracic anterior thoracotomy (Chamberlin procedure) have been performed successfully in four children under local anesthetic as young as 10 to 12 years of age. This can be done with the children in the semiupright position with spontaneous ventilation. The partially upright position will maximize the compromised respiratory function, as has been shown in the previous studies of pulmonary function.[22] Spontaneous ventilation minimizes collapse of the trachea by the negative pressure exerted by the chest wall. By following these guidelines for the use of general or local anesthesia, and the biopsy techniques described, a safe biopsy can be obtained on essentially all children and adolescents with an anterior mediastinal mass ( Fig. 12-4).

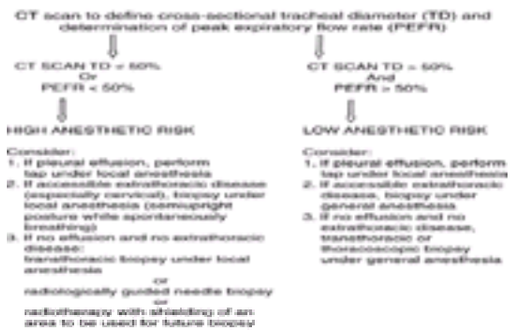


**FIGURE 12-4.** Algorithm for management of a child or adolescent presenting with an anterior mediastinal mass. CT, computed tomography.

### *Management of Pheochromocytomas*

Pheochromocytomas in children generally present with hypertension and associated symptoms.[27] Once the diagnosis is confirmed by a 24-hour urine collection for catecholamines and localization is performed, surgical removal is the definitive therapy. The principles of preoperative management are blood pressure control and the repletion of intravascular volume. Alpha-adrenergic blockade should begin at least 1 week before the surgery and is usually accomplished through the use of phenoxybenzamine. This is a long-acting alpha-adrenergic antagonist that is administered orally and is usually well tolerated by children. The usual starting dose for phenoxybenzamine is 0.25 mg per kg per day divided into a twice-a-day dose. The amine synthesis inhibitor metyrosine may be effective in children with hypertension unresponsive to phenoxybenzamine. The intravenous administration of phentolamine, with its short half-life, may on occasion also be useful in the control of bouts of hypertension. Beta-adrenergic blockade should be reserved for the treatment of persistent sinus tachycardia and arrhythmias associated with prior alpha-blockade and should never precede alpha-blockade. Adequate intravascular volume expansion is required preoperatively, as patients with pheochromocytoma often manifest reduced intravascular fluid and decreased red cell mass. The surgical removal of the pheochromocytoma is coincident with a drop in circulating catecholamines and possible hypotension, which may require further fluid administration. Conversely, the induction of anesthesia or the manipulation of the tumor(s) during resection can result in hypertensive episodes that are best treated with intravenous sodium nitroprusside.

## CANCER SURGERY

### Staging

It is critical to establish the correct stage of a tumor for optimal treatment of pediatric solid tumors. All protocols are based on stage of the tumor, which determines such factors in treatment as use and extent of radiotherapy and intensity of chemotherapy. In an era when cure can be achieved in the majority of patients, efforts to limit therapy to minimize the long-term sequela of treatment assume a prominent role. Correct staging is essential to our ability to minimize therapy and yet maximize cure. It has been demonstrated in several tumors that treatment of children based on inadequate or incorrect staging results in an increased incidence of relapse.[28,29] Only in the management of Hodgkin's disease has surgical staging decreased in importance as systemic therapy has been uniformly used.

A unique staging system is used for each of the solid tumors, and these will be presented in the appropriate chapters. It is important to note that these staging systems are not static tools, but that they have evolved over time as the significance of specific criteria for staging and treatment have been established. Hilar lymph node involvement in Wilms' tumor was initially considered as a criterion for stage II in the initial two National Wilms Tumor Study Group (NWTSG) protocols. Based on the demonstrated increased incidence of local recurrence in children with hilar lymph node involvement in the National Wilms Tumor Study-2, hilar lymph node involvement became a criterion in subsequent studies for stage III.[30,31] A new staging system for neuroblastoma, the International Neuroblastoma Staging System (INSS), was created in the 1990s by a committee of international experts on neuroblastoma based on the results of prior studies.[32] The treatment for neuroblastoma has progressed even further of late so that not only stage, but the biologic markers of the tumor now determine the intensity of treatment in many protocols.[33]

The surgeon is critical to the proper assignment of stage. A surgical staging system is used for most tumors. In these systems, the extent of residual disease after resection as well as lymph node involvement define the stage. In Wilms' tumor, lymph nodes must be sampled from not only the perihilar region, but from along the aorta or vena cava to adequately stage the child. It is of note that it has been well established in Wilms' tumor that assessment of lymph node involvement by gross inspection has a very poor correlation with the histology of the node. Othersen and his colleagues demonstrated a false-negative rate of 31.3% and a false-positive rate of 18.1% in a prospective series.[34] In a similar review by Jereb and associates, a false-positive rate of 72% and a false-negative rate of 7% occurred in a series of International Society of Paediatric Oncology (SIOP) patients.[35] It was demonstrated in a more recent review from NWTS-4 that an increased incidence of local recurrence occurred in children in whom lymph node sampling was not performed. The recurrence rate was actually higher than that of children with hilar lymph node involvement who had been appropriately treated.[28] The reason for the increased local recurrence was presumed under treatment of the children; the increased risk of recurrence occurred primarily in children with stage I disease who receive minimal chemotherapy and no radiotherapy.

The new INSS is also a surgical staging system in which the size of the tumor, extent of residual disease, and involvement of ipsilateral and contralateral nonadherent lymph nodes define the stage.[32] Again, the gross assessment of lymph node involvement is not adequate for evaluation. Wilson and associates reported a sensitivity of 76% and a specificity of 77% for lymph node involvement by neuroblastoma based on gross inspection of the nodes.[36]

The staging of rhabdomyosarcoma is complex because of the multiple primary sites of involvement. Response of this tumor to therapy is very site specific. Rhabdomyosarcoma is staged on both tumor, node, metastases (TNM) and clinical group systems by the Intergroup Rhabdomyosarcoma Study (IRS). The TNM system is thought to best define the pretreatment extent of disease and allow comparison between results of various studies.[37] The clinical group system is a surgical staging system in which the extent of resection and the presence of nodal involvement are prime components. An initial incisional biopsy should be performed in most cases of suspected rhabdomyosarcoma. The biopsy site and direction of the incision should always be planned with future resection in mind. Injudicious attempts at initial resection of an extremity lesion, leaving tumor at the margins of resection, can greatly complicate future resection.[38]

The need for lymph node biopsy in rhabdomyosarcoma is determined by the primary site. Children presenting without evidence of distant metastases have an overall 10% incidence of lymphatic spread. The frequency is highest for primary lesions arising in the prostate (41%), paratesticular (26%), and genitourinary sites (24%).[39] Extremity lesions have an intermediate frequency (12%) whereas nonorbital head and neck sites (7%), truncal sites (3%), and the orbit (0%) had the lowest frequency. In extremity and genitourinary sites, it is particularly important to establish lymph node involvement to allow appropriate design of radiation fields. A representative sample of lymph nodes from the draining nodal group should be biopsied in these lesions. A lymph node resection should not be performed, however, because it may produce lymphedema, which will complicate radiotherapy and subsequent surgical resection of the primary lesion.

Radiologic evaluation of lymph node involvement has been demonstrated to have a low sensitivity. A total of 121 boys with paratesticular rhabdomyosarcoma treated

on IRS III had a retroperitoneal lymph node dissection to evaluate nodal status. [40] Lymph nodes were assessed to be negative based on CT in 18% of the boys, 14% of whom had positive nodes when biopsy or retroperitoneal lymph node dissection was performed. Of the clinically positive boys, 94% were confirmed to be positive pathologically. Retroperitoneal relapse occurred in only 2 of the 121 boys, one of whom had pathologically negative lymph nodes and did not receive radiotherapy. Although CT was very accurate if lymph node abnormalities were identified, it was not extremely sensitive in identifying nodal involvement. In a subsequent study, Wiener and colleagues from the IRS have reported an increased incidence of retroperitoneal relapse in children treated during IRS IV. [29] In this study, the use of abdominal irradiation was based on thin-cut CT scans in 98% of cases as compared with children treated during IRS III in which 94% had retroperitoneal biopsy or lymph node dissection. A decrease in stage II disease (positive lymph nodes) was seen between IRS III and IV from 35% to 17%, with a corresponding decline in the use of irradiation. This resulted in a fourfold increase in retroperitoneal lymph node recurrence. This is another example of increased local failure resulting from inadequate staging.

Regional lymph node involvement is an extremely important prognostic variable in children with extremity rhabdomyosarcoma. In one study by Mandell and associates, excluding children with metastatic disease, survival in those without nodal involvement (11 of 12) was significantly better than in those with lymph nodes involved with tumor (one of ten; $p = .001$).[41] A similar finding to that in Wilms' tumor was demonstrated in children who had a distal extremity rhabdomyosarcoma with no evidence of distant metastasis. Survival was significantly better in those with biopsy-proven negative nodes than in those in whom nodes were not biopsied. [38] These findings highlight the essential nature of proper staging to achieve maximum survival.

### Biopsy

Use of a correct biopsy technique is critical to a child with a solid tumor. Fundamental considerations when selection of the method and incision are made must include (a) creation of an incision that may be incorporated in the future incision used for resection, (b) avoidance of contamination of an uninvolved body cavity, (c) avoidance of contamination of otherwise uninvolved lymphatic drainage, and (d) adequate staging must be accomplished at the time of biopsy if the child is to receive preoperative chemotherapy.

Some tumors will be appropriate to biopsy by percutaneous needle technique often with ultrasound guidance. Examples are chest wall tumors, metastatic neuroblastoma, and extensive hepatic tumors. The definitive diagnosis in these lesions can be obtained in most cases with adequate material obtained for both cytogenetic studies and biologic markers, which are critical to the complete characterization of solid tumors. [42,43] The primary tumors on which needle aspirate or core biopsy may fail to provide the diagnosis are the lymphomas, particularly Hodgkin's disease. In many of these cases, an incisional biopsy will be required to obtain a conclusive diagnosis.

In most extremity lesions in which the benign or malignant nature of the tumor is not known, a preliminary incisional biopsy is important. An injudicious attempt to remove the lesion with narrow margins may require a more extensive resection to obtain adequate margins than if the resection had been performed after an incisional biopsy. The importance of obtaining negative microscopic margins in rhabdomyosarcoma has been well established by Andrassy and his colleagues in the IRS.[39] A longitudinal incision is optimal in almost all extremity lesions. It will allow the scar to be easily removed when the definitive resection is performed. A transverse scar will require a larger amount of the skin to be resected.

Examples of avoiding contamination of an uninvolved body cavity at biopsy are Ewing's sarcoma of the chest wall and Wilms' tumor. Percutaneous or open biopsy of a chest wall Ewing's sarcoma will avoid the pleural cavity being contaminated as would occur if a thoracoscopic biopsy were performed. Contamination of the pleural cavity in most cases of Ewing's sarcoma will necessitate its irradiation. In most cases of Wilms' tumor, it is best to resect the primary without a preliminary biopsy. This will avoid potential extensive contamination of the abdominal cavity, which would advance the child to stage III and on NWTSG protocols would require the use of irradiation to the abdomen. The infrequent exceptions to this policy are discussed in the section Wilms' Tumor.

An important example of avoiding contamination of an uninvolved lymphatic system occurs in boys with testicular masses. The vital importance of performing a transinguinal biopsy and not a transscrotal biopsy has been long stressed. A transinguinal approach allows proximal control for high ligation of the spermatic cord, which is the route of both lymphatic and hematogenous extension from the testes. A transscrotal approach will contaminate the scrotum, which has lymphatic drainage via the inguinal and iliac system. This approach also does not provide access to the proximal spermatic cord for high ligation.

## PREOPERATIVE ADJUVANT THERAPY

The role of preoperative adjuvant therapy has been established based on completed studies, and its use is very disease specific. Several important questions must be addressed when preoperative therapy is considered. First, will information regarding stage or histology be lost, adversely affecting ultimate therapeutic decisions? Second, will children receive chemotherapy who do not otherwise require it? Third, will adjuvant therapy facilitate surgical resection and decrease the risks of complications? These issues are considered for several of the solid tumors.

### Wilms' Tumor

The two principal multiinstitutional groups with therapeutic trials in Wilms' tumor have adopted quite different approaches to the use of adjuvant therapy. Primary nephrectomy has been recommended for patients with Wilms' tumor by the NWTSG. In contrast, initial chemotherapy has been used extensively by members of the SIOP. The potential benefit of preoperative therapy must be balanced against the disadvantages. Treatment without any biopsy has been difficult to support when both NWTSG and SIOP series have reported a 7.6% to 9.9% rate of benign or other malignant diagnosis in children with a prenephrectomy diagnosis of Wilms' tumor.[44,45 and 46] Zuppan and associates have shown that the histologic diagnosis after preoperative treatment in a group of children followed on NWTSG studies did not appear to have been significantly distorted by pretreatment, but loss of evidence of lymph node involvement has been of significant concern. [47] “Downstaging” of tumors was seen in two consecutive but nonrandomized SIOP studies in which the proportion of low-stage disease increased after preoperative therapy when compared with earlier studies using primary surgery. These findings suggested that the preoperative treatment significantly decreased the apparent stage of the children.[48] The proportion of stage I patients at surgery rose from 22% to 48% after chemotherapy. The third SIOP study of Wilms' tumor randomized the use of local radiotherapy (20 Gy) in children treated preoperatively with chemotherapy (vincristine and dactinomycin) who had stage II lymph node–negative disease at resection. All children received vincristine and dactinomycin for 38 weeks. A high rate of stage I tumors (52%), was found in this study, with a low frequency of ruptures (7%). The study was terminated after randomization of 123 children because of an increased incidence of abdominal recurrence during the first year of follow-up in the children not receiving radiation (6 of 59 versus 0 of 64). [49] This suggested that prenephrectomy treatment altered the pathologic findings, which would have led to a diagnosis of stage III disease (i.e., lymph node involvement) and to the standard administration of local irradiation. The absence of this finding in the posttherapy specimens, however, did not obviate the apparent need for radiotherapy to prevent local recurrence.

A major driving force for the use of preoperative therapy by SIOP was the high rate of operative tumor rupture in their early series employing primary resection of the tumor. The rupture rate decreased from 33% (20 of 60) to 4% (3 of 72) with preoperative abdominal irradiation (20 Gy) in the first randomized SIOP study. [45] It must be noted, however, that 33% is an extremely high frequency of rupture. Survival was not affected, and the incidence of local recurrence was not reported. In NWTS-1 and -2, operative rupture occurred in 22% and 12% of children, respectively. [44,50] In a subsequent SIOP randomized study, the rate of rupture was essentially the same for children receiving abdominal irradiation (20 Gy) and actinomycin D (9%, 7 of 76) and those receiving vincristine and actinomycin D (6%, 5 of 88). [45]

A second consideration in the use of preoperative chemotherapy in Wilms' tumor has been whether it will allow the safe performance of partial nephrectomy in some cases. This has been evaluated in several centers. McLorie and associates in Toronto obtained percutaneous biopsy in 37 children with Wilms' tumor and then administered multi-agent chemotherapy for 4 to 6 weeks. A partial nephrectomy was then performed in nine children (four with unilateral and five with bilateral tumors).[51] Two children experienced intraabdominal relapse. Only 4 of the 30 unilateral tumors (13.3%) were amenable to a partial nephrectomy. Another analysis of the feasibility of partial nephrectomy was performed at St. Jude Children's Research Hospital. [52] Preoperative computed tomograms (CT scans) of 43 children with nonmetastatic unilateral Wilms' tumor were reviewed retrospectively. Criteria to allow partial nephrectomy were involvement by the tumor of only one pole and less than one-third of the kidney, a functioning kidney, no involvement of the collecting system or renal vein, and clear margins between the tumor and surrounding structures. Utilizing these criteria, only 2 of 43 scans (4.7%) suggested that partial nephrectomy was feasible.

The primary concern regarding use of preoperative chemotherapy, as reported in the studies of Cozzi and Moorman-Voestermans and their colleagues, to shrink small tumors to allow partial nephrectomy is that these children may be curable by surgical resection alone without subjecting them to the toxicity of additional treatments.[53,54]

Preoperative treatment of Wilms' tumor is generally accepted in certain instances. These include the occurrence of Wilms' tumor in a solitary kidney, bilateral renal tumors, tumor in a horseshoe kidney, a massive tumor that would require resection of other involved organs, and respiratory distress from extensive metastatic tumor. Pretreatment biopsy should be obtained. The aim of treatment before surgical resection in the bilateral tumors and when tumor occurs in a solitary kidney is to preserve maximum renal tissue and function. In the NWTSG review of the 55 children who developed renal failure treated on NWTS-1 to -4, 39 had bilateral tumor involvement. Increasing efforts to preserve renal parenchyma in bilateral cases in the series of NWTSG studies resulted in a decline in the incidence of renal failure from 16.4% in NWTS-1 and -2 to 9.9% in NWTS-3 and 3.8% in NWTS-4. [55] In most cases, preliminary treatment after biopsy and staging produces a significant decrease in size of the tumor and facilitates resection of the tumor with preservation of a portion of the kidney. One hundred and thirty-four kidneys in 98 children with bilateral Wilms' tumors were managed with renal salvage procedures during NWTS-4. [56] Complete resection of gross disease was accomplished in 118 kidneys (88%). A higher incidence of positive surgical margins (16%; 19 of 134) and local tumor recurrence (8.2%; 11 of 134) was seen in this group of children than in those with complete nephrectomy. This was justified, however, by the desire to preserve renal tissue and thus avoid renal failure. Overall, portions of 72% of the kidneys were preserved, and the 4-year survival rate was 81.7%.

A final indication for preoperative therapy in Wilms' tumor may be in children with intravascular extension of the tumor. Some studies have demonstrated a decreased incidence of complications in children with atrial extension receiving preoperative chemotherapy compared with children with primary surgical resection. [57]

**Neuroblastoma**

The surgical approach to neuroblastoma is determined to a great extent by the size and location of the tumor. Many low-stage tumors with favorable biologic markers will be cured by surgery alone, even with positive microscopic margins or residual tumor. [58] Regrettably, the vast majority of children with neuroblastoma presents with large stage 3 primaries that cross the midline or with metastatic disease. Consideration of preoperative adjuvant therapy is appropriate in these cases. All of these children will require adjuvant chemotherapy, so its administration before resection will not subject them to treatment they would not otherwise receive. Biopsy and staging can generally be achieved with limited surgical procedures and radiographic demonstration of distant metastasis if they are present. It is critical that adequate tissue be obtained to allow evaluation of the histology and biologic markers to fully characterize the tumor.

Large primary tumors often encircle critical vessels such as the celiac axis, the superior mesenteric artery and vein, and the renal vessels. Although two studies have shown no difference in the surgical complication rate between initial and postinduction resection, [59,60] others have demonstrated a higher incidence of complications, including nephrectomy, in the group undergoing initial resection. [61,62,63] and [64] Preoperative chemotherapy decreases the vascularity and friability of the tumor and facilitates resection, particularly in developing a dissection plane between the tumor and the great vessels ( Fig. 12-5).[65] Preoperative therapy may be of particular importance to preservation of renal function. Many current protocols for children with stage 4 disease have significant nephrotoxicity, and loss of a kidney from surgery can significantly limit the intensity of therapy. A multiinstitutional review of children treated over an 11-year interval demonstrated a 14.9% (52 of 349 children) incidence of nephrectomy or renal infarction during surgery for local control. [64] There was a 25% incidence among those with initial resection (29 children) and a 9.9% incidence in the post-chemotherapy resections (23 children). In children undergoing initial resection, the risk of nephrectomy was more than twice that compared with those undergoing resection after chemotherapy ($p$ = .012). Hence, the approach for neuroblastoma regarding primary therapy of large lesions is quite different from that in Wilms' tumor.

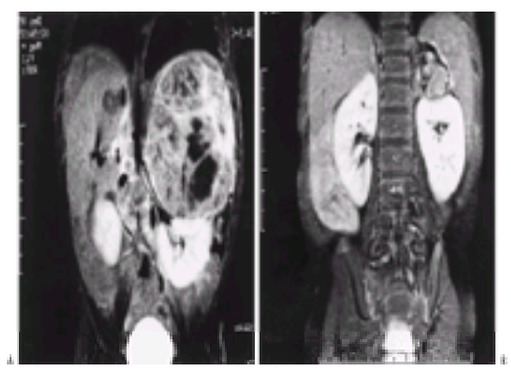

**FIGURE 12-5. A:** This magnetic resonance imaging scan of a 9-month-old boy with neuroblastoma who presented with a palpable left abdominal mass shows a large left adrenal tumor. The child was not found to have metastatic disease, but did have N- *myc* amplification. **B:** Dramatic response of the tumor to adjuvant chemotherapy is shown in the follow-up scan.

**Ewing's Sarcoma**

Another tumor in which preoperative adjuvant chemotherapy has been found to be of benefit is Ewing's sarcoma/primitive neuroectodermal tumor. Adjuvant therapy is required for all children with this diagnosis because of the very high incidence of local and distant relapse without such therapy. Its use preceding surgery has been of benefit in decreasing the size of the tumor as well as its friability and extremely vascular nature ( Fig. 12-6).[66] Recent multiinstitutional studies of children with chest wall primaries have demonstrated a major benefit of preoperative therapy. Complete resection with negative pathologic margins is more frequently achieved in children who have received preoperative chemotherapy. [67] This is of particular importance for children with chest wall primaries because they can then avoid the use of radiotherapy to the chest with its attendant risks of pulmonary and cardiac injury.

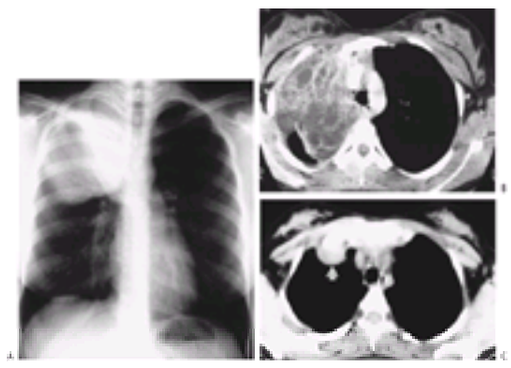

**FIGURE 12-6. A:** Chest radiograph of a 14-year-old girl with Ewing's sarcoma, who presented with a cough, reveals a mass in the apex of the right chest. **B:** Computed tomography showed a large mass of varying density without bone erosion. Biopsy was achieved by percutaneous technique. The chest radiograph after 2 months of therapy was normal, and computed tomography **(C)** showed a small residual soft tissue mass (*arrow*). No viable tumor was found in the mass on pathologic examination after resection.

**Hepatoblastoma**

Resection remains critical in the cure of hepatoblastoma. Even with current multi-agent chemotherapy, cure is rarely accomplished without successful resection. There have been multiple reports from single institutions describing the benefit of preoperative doxorubicin or cisplatin–based chemotherapy converting “unresectable” tumors into tumors that can be safely resected ( Fig. 12-7).[68,69,70,71] and [72] Several prospective multiinstitutional trials have also supported the role of adjuvant chemotherapy in shrinking large “unresectable” hepatoblastomas. King and associates reported the experience from the Children's Cancer Group protocol,

which included both hepatoblastoma and hepatocellular carcinoma.[73] In that study, 34 children were deemed to be “unresectable.” Twenty had successful resection after preliminary therapy. There was no difference in survival between children resected initially and those with delayed resection, but a higher incidence of surgical complications was seen in children with delayed resection. In a similar study reported by Reynolds and colleagues from the Pediatric Oncology Group, 37 children with “unresectable” tumors received preoperative therapy.[74] Twenty-nine of those responded to chemotherapy with a cisplatin-based regimen, and 26 underwent delayed surgical resection. An increased incidence of surgical complications was again seen in those children with delayed resection, but the overall survival of the primary resection and delayed resection groups remained equivalent. Results from the German Cooperative Pediatric Liver Tumor Study were reported by von Schweinitz and coauthors.[75] Chemotherapy reduced the size of the tumor in 33 of 37 children (89%) with hepatoblastoma. This study identified a potential risk to a prolonged course of preliminary chemotherapy. Drug resistance developed in 6 of 11 children treated with four or more courses before resection. It was demonstrated by regrowth of the primary tumor and rise in the serum alpha-fetoprotein (AFP) levels.

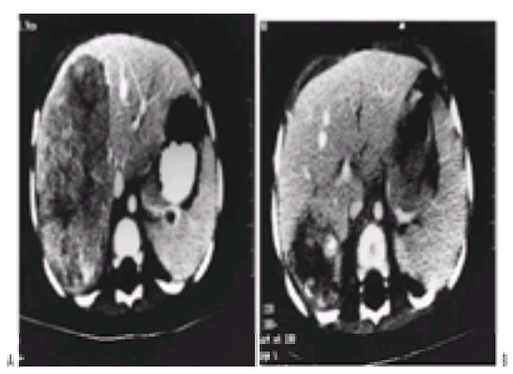

**FIGURE 12-7. A:** Computed tomogram of a 16-month-old boy who presented with a palpable upper abdominal mass. Percutaneous biopsy confirmed the diagnosis of hepatoblastoma. **B:** Tomogram after four cycles of cisplatin-based chemotherapy revealed excellent response to treatment; a formal right hepatic lobectomy achieved complete resection of the tumor.

Postoperative chemotherapy will be utilized in most children with hepatoblastoma because of the risk of distant relapse. Preliminary treatment should therefore be considered after biopsy in a child with a large primary that the surgeon thinks would present a significant risk for resection. Preoperative therapy should not be continued, however, beyond the third or fourth course if possible because of the potential development of drug resistance.

## MINIMALLY INVASIVE SURGERY

Over the past decade, minimally invasive surgical techniques using laparoscopic and thoracoscopic techniques have become common practice. Improved cameras, new trocar systems, and specifically designed instruments now allow a wide spectrum of operations to be performed without the use of large thoracotomy or laparotomy incisions. In the realm of pediatric surgical oncology, these methods have been most broadly applied to biopsies and the excision of selected small masses. A CT scan or magnetic resonance imaging is very helpful in planning the appropriate surgical approach. Oncologic surgical principles, such as adequate exploration, complete excision of a mass with an appropriate margin, and minimizing the risk of a tumor spill, are still paramount. Although rare and possibly technique-related, trocar site tumor implantation has been reported.[76,77]

Thoracoscopic surgery is usually performed under general anesthesia in a full lateral position to allow for visualization of all the lobes of the lung. Nodules that are located peripherally in the lung, along the parietal pleura, or on the diaphragm are particularly suitable for biopsy. In larger patients, a double lumen endotracheal tube allows for selective lung ventilation and, hence, permits more facile surgery. Thoracoscopic surgery to excise small tumors of the lung is frequently accomplished with the aid of an intracorporeal stapling device. Although thoracoscopy is often suitable for the removal of secondary lung tumors, osteogenic sarcoma metastatic to the lung is usually best managed by standard thoracotomy. This is because thoracoscopy and CT scan often miss small intraparenchymal osteogenic sarcoma secondaries that may be easily palpated. The mediastinum is also accessible by minimally invasive techniques and excisional or incisional biopsies, or thoracoscopically guided needle biopsies may be performed.

Laparoscopic surgery is performed under general anesthesia and relies on $CO_2$ insufflation of the peritoneal cavity to permit visualization. Staging laparoscopy and biopsies of primary and secondary tumors have frequently been performed. If oncologic principles are not compromised, tumor resection may also be considered in selected cases. Laparoscopic splenectomy, nephrectomy, adrenalectomy, lymph node dissection, and colectomy have all been performed in patients with malignancies. At present, no large-scale prospective trials to evaluate these approaches have been completed.

## SPECIFIC TUMOR CONSIDERATIONS

### Wilms' Tumor

Resection continues to play a major role in the local control of Wilms' tumor. The importance of complete staging has already been stressed. In most cases, except those previously discussed, the initial management of a renal mass is nephrectomy. Despite the fact that most Wilms' tumors present as a large mass, safe resection is generally feasible. Wilms' tumor, in contrast with neuroblastoma, is less likely to directly invade surrounding organs, complicating resection. It is important that an appropriately sized transverse upper abdominal or thoracoabdominal incision be used. A flank incision does not allow examination of the contralateral kidney or adequate lymph node biopsy, and a small incision in all too many cases results in rupture of the tumor and requires subsequent abdominal irradiation.

The vital importance of lymph node biopsy has already been stressed. There is no role, however, for an extensive lymph node resection, as this has not decreased the incidence of local recurrence or improved long-term, event-free survival.[34,35] One additional consideration during resection of Wilms' tumor is intravascular extension. This has been documented to occur in 4.1% to 6.0% of children treated on NWTS-3 and-4, respectively, and it should always be suspected.[78] Preoperative imaging should be evaluated for evidence of intravascular extension, but it is not invariably found. Before ligation of the renal vein, it should be palpated to make certain that there is not intravascular extension of the tumor that might embolize with ligation of the vein. Second, if unexplained hypotension occurs during the course of nephrectomy, the possibility of a pulmonary embolism must always be entertained. Current recommendations from the NWTSG are that children with intravascular extension to the atrium or inferior vena cava extension to the level of the hepatic veins should have a biopsy of the tumor followed by chemotherapy before resection of the tumor.

### Neuroblastoma

Neuroblastoma is an enigmatic tumor in its clinical and biologic behavior; on the one hand, it is a tumor characterized by spontaneous regression, but, in contrast, it is commonly present in a clinical form resistant to all forms of antitumor therapy, with a result being little improvement in disease prognosis over the past several decades. The most frequent locations for neuroblastomas include the abdomen in suprarenal or paraaortic sites and, less frequently, in a thoracic paraspinal location or along the cervical sympathetic chain. Pelvic and retro-orbital intracranial locations have also been reported as sites for this neural crest tumor. Neuroblastomas have a propensity to invade surrounding tissues, especially enwrapping nerves and vessels in the renal hilum and pancreatic areas, and in the paraspinal location there is a unique characteristic of this tumor to grow through vertebral foramina into the spinal canal itself.[79]

Clinical neuroblastomas are characterized by several unique features that may influence operative therapies. These tumors metabolize catecholamines and as a result excrete various markers of catecholamine metabolism, and therefore tumor activity, including vanillylmandelic acid or homovanillic acid, and quantitative ratios can indicate tumor differentiation and can impact on tumor prognosis. Such catecholamine marker excretion is used for mass population screening. Neuroblastomas also have the propensity to spontaneously differentiate, or they can be stimulated to differentiate from a malignant neuroblastoma into a benign ganglioneuroma. Such latter tumors potentially can occur anywhere a neuroblastoma might be found, but they most commonly are located in the thoracic paraspinal region. The most unusual behavior of neuroblastoma is its propensity for spontaneous regression. This predilection is seen in two unique tumor populations. First, there is the mass infant population neuroblastoma-screened patients in whom a high incidence of stage 1 tumors are found, which seem to have a characteristic “benign” and even

regressive pattern. A second group is the child typically younger than 1 year of age who presents with a primary tumor as well as evidence of tumor in the skin, liver, and bone marrow, the so-called Evans stage IVS. In approximately three-fourths of such patients, with or without treatment directed at the primary tumor, spontaneous regression of the neuroblastoma found in these sites occurs, and recovery and cure is the outcome. [2]

The role of operation in neuroblastoma remains controversial. Although complete resection of the tumor is associated with the best survival in patients with localized neuroblastoma, the role of operation may be less important in the care and treatment of most neuroblastomas. In fact, the value of complete tumor removal in localized neuroblastomas may be overestimated, as the ability to resect the tumor may be an expression of a favorable biology of the tumor with a propensity to either spontaneously regress or clinically behave less aggressively. [80] In fact, the mass screening findings of localized neuroblastomas as currently reported in Japan has resulted in a portion of such patients being treated by observation only, without resection, and the outcomes seem quite favorable. [81] The role of operation is uniquely beneficial in selected patients with compressive symptoms, including those with spinal cord compression, mediastinal distortion, celiac axis enwrapment, or even renal artery encasement with secondary hypertension. On rare occasion, hollow visceral compression results in symptoms of obstruction. Because more than one-half of the patients at the time of presentation with neuroblastoma have either advanced local or metastatic disease, multimodal therapy is an absolute requirement for the total management plan. Because the definitive treatment plan for neuroblastoma is influenced to a great degree by cytogenetics, tumor oncogene amplification, and histology, the role of an adequate surgical biopsy specimen to help define an appropriate treatment plan cannot be underestimated.

Though there is substantive experimental evidence that a variety of surgical adjuncts, such as electrocauterization or laser therapy, may be immune response enhancing for a tumor known to be potentially influenced by an augmented host tumor relationship, the benefit of such techniques to the removal of clinical neuroblastoma is less certain.[79] The application of minimally invasive surgery in the resection of neuroblastomas is in its infancy, and to date minimally invasive technique has largely been confined to a role for tumor biopsy from primary or metastatic sites and as an adjunct to tumor staging.

The principles of operative therapy for neuroblastoma are largely stage related ( Fig. 12-8). For INSS stage 1, a complete resection of localized neuroblastoma may be the only therapy required. Regional lymph nodes should be excised during operation for accurate staging, but adjacent normal organs should be spared. After complete operative resection, observation is the only required follow-up.

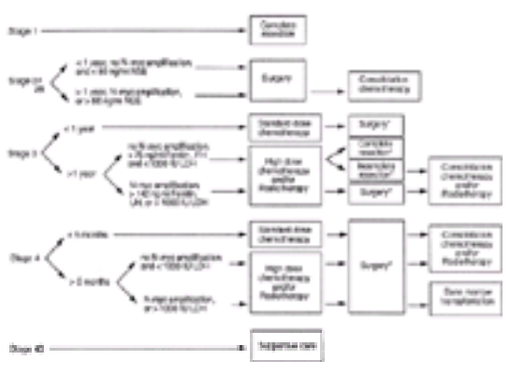

**FIGURE 12-8.** Schema for operative intervention in neuroblastoma. *Second look or delayed primary surgery. FH, favorable histology; LDH, lactic (acid) dehydrogenase; NSE, neuron-specific enolase. [Modified from Ishizu H, Ziegler MM. Neoplasms. In: Levine BA, Copeland EM, Howard RJ et al. (eds). Current practice of surgery. New York: Churchill Livingstone, 1992;3.]

For INSS stage 2A and 2B, such localized neuroblastomas are also treated to achieve complete operative resection. Because of microscopic residual disease, however, second look or even delayed primary operation may be planned after tumor reduction with chemotherapy or radiation therapy, or both. Lymph node resection is beneficial for reducing tumor burden as well as precisely staging the disease, and depending on associated risk factors, such as age, N- *myc* amplification, histology, serum ferritin, and neuron-specific enolase, adjunctive chemotherapy will likely be considered.

For INSS stage 3 tumors, chemotherapy is often the first treatment, and delayed primary or second look resection may be scheduled after initial therapy. At delayed operation, total tumor removal or debulking (cytoreduction) may be done, taking care to preserve adjacent normal organs. Almost three out of four localized neuroblastomas previously deemed unresectable will be able to be resected after induction chemotherapy. [82]

For INSS stage 4 metastatic neuroblastomas, aggressive multimodal therapy is indicated to improve an otherwise dismal outcome. Primary or delayed resection is of uncertain benefit in metastatic neuroblastoma. There are data that suggest a favorable benefit of complete resection of the primary tumor as well as involved regional lymph nodes, but there are also contrasting data that suggest no favorable impact on outcome of these resections. Cytoreduction seems preferable to biopsy alone, however, and surgical resection with removal of as much tumor as possible is clearly beneficial for those patients who are eventually treated by autologous or allogenic bone marrow transplantation after high-dose chemotherapy and radiation. [83]

Perhaps most controversial is the treatment of INSS stage 4S. This tumor group may best be observed only with the use of supportive therapy while awaiting spontaneous regression. Because selected infants present with life-threatening hepatomegaly and intraabdominal compression, either minimal resection and/or auxiliary procedures, such as operative placement of a silastic pouch on the abdominal wall to expand the cavity, may be indicated to control diaphragmatic elevation and respiratory distress or vena caval obstruction. [84] Such palliative procedures carry an extensive risk of infection and local wound problems that may further complicate the clinical course of infants with this rapidly changing tumor, which, eventually, if time can be spared, will spontaneously regress in three out of four cases. In series comparing resection versus no resection of the primary tumor in stage 4S patients, little difference in outcome is noted; but what is less clear is the role of aggressive operation for those stage 4S patients with adverse tumor markers. Finally, for patients with localized neuroblastoma found by neonatal screening, observation alone may be most appropriate operative therapy for stage 1 disease, and a current multiinstitutional prospective study is evaluating this hypothesis.

Neuroblastomas that penetrate the spinal canal or that enwrap vascular structures have to be individualized in their operative management. When children present with spinal cord compression, immediate spinal cord decompression is indicated by either laminectomy or radiation therapy. Transthoracic resection of the posterior paraspinal component of such tumors requires the removal of the majority of tumor with an effort to remove its intravertebral dumbbell extensions in a conservative yet as complete a fashion as is possible. Certainly, intraspinal bleeding or cerebral spinal fluid leak must be avoided. Residual foraminal disease typically does not carry an adverse prognosis. Those tumors enwrapping vascular structures, such as the celiac axis or renal vessels, or both, are best treated not by en bloc removal with vascular reconstruction, but rather by tumor separation, division, and vascular dissection with preservation such that the tumors at times are removed piecemeal. This operative technique is based on the premise that such patients require adjunct therapies for disease control and cure after their operation.

Tumor outcomes (Fig. 12-8) are largely a product of tumor stage, patient age, and various biochemical and oncogenic markers. Other than those exceptions noted, there is not substantive data available suggesting the advantage of one operative technique versus another with an influence on tumor outcome because operative resection is coupled with multimodal antitumor therapy. Tumor resection of massive celiac axis or pararenal neuroblastomas, or both, at times results in the unavoidable removal of an adjacent kidney, a not preferred outcome in patients eventually facing bone marrow transplantation. Outcomes for patients after resection of high intrathoracic paraspinal neuroblastomas almost routinely includes the development of an ipsilateral Horner's syndrome because tumor removal requires resection of the sympathetic chain and the stellate ganglion. [85] Whether there is advantage in patient outcomes from the application of minimally invasive surgical removal of localized neuroblastoma is speculative, but early reports suggest shorter lengths of stay and less perioperative morbidity when such techniques are applied.

**Hepatoblastoma**

Malignant liver tumors in infancy and early childhood typically are hepatoblastomas, whereas in older adolescents or those children with metabolic derangement or postviral illness, the tumor histology is typically a hepatocellular carcinoma. Despite further refinement in adjuvant therapies, the goal of operation remains clean-margin tumor resection if any opportunity for cure is to be achieved. Therefore, once the tumor in the liver is found, the initial main step is accurate preoperative imaging assessment to determine the optimal operative approach.

For tumor volumes and location that encroach or cross the anatomic plains that define lobectomy or extended lobectomy, or for multicentric bilobar tumors, preoperative chemotherapy may be optimal.[86,87] For resistant tumors, innovative therapies have included intraarterial chemotherapy or even chemoembolization. [88,89]

Hepatic lobectomy is the preferred operation for primary liver tumors, and though technically more exacting, an extended right or left lobectomy (trisegmentectomy) may also be indicated.[90,91] Nonanatomic resections that cross anatomic plains at times become a technical necessity when removing large tumors deep within the substance of the liver and for postresection locally recurrent disease. The need for perioperative chemotherapy and the extent of resection, at least in experimental animals, does not influence postoperative liver regeneration. [92] Finally, for those primary or recurrent tumors not amenable to resection, yet whose disease extent is confined to the liver, total hepatectomy followed by liver transplantation is a therapeutic alternative, though eventual disease-free outcomes are suboptimal. [93]

**Rhabdomyosarcoma**

Rhabdomyosarcoma is a tumor of mesenchymal origin that can present in almost any site but most commonly is found in the head and neck (35%), genitourinary tract (26%), and limbs (19%).[94] Approximately 250 new cases of pediatric rhabdomyosarcoma are diagnosed annually in the United States. The embryonal pathologic type is the most common form of rhabdomyosarcoma and in general has a better prognosis than the alveolar type. Although histology is an important predictor of survival, tumor site and the presence of distant metastases are even more significant determinants. Tumors of the orbit, superficial head and neck, testes, vagina, and uterus all have 4-year survivals of approximately 90% whereas tumors of the parameningeal area, bladder, prostate, and limbs have substantially lower survival rates that approximate 65%.

The successful treatment of this diverse group of tumors often requires multimodal therapy, including chemotherapy, radiotherapy, and surgery. The tissue diagnosis of rhabdomyosarcoma is usually made by surgical biopsy, although needle biopsy is considered in selected cases if surgical biopsy is deemed to have too high a morbidity. Surgical biopsy ensures that adequate tissue is available for histochemical and cytogenetic analysis. If the tumor is present in an extremity, care must be taken to place the initial biopsy along the long axis of the limb to allow for subsequent excision with an adequate margin. Effective surgical therapy of tumors in most sites consists of complete resection of the malignancy with pathologic margins that are free of tumor. If microscopic residual remains after an attempted curative resection, re-excision does appear to proffer a survival benefit. [95,96] Neoadjuvant chemotherapy followed by surgery may sometimes be used advantageously to preserve organ function and cosmesis while allowing for adequate oncologic surgery. Debulking procedures are not indicated.

**Germ Cell Tumors**

Pediatric germ cell tumors are rare. They occur in the gonads (testes and ovaries) as well as extragonadal sites (sacrococcygeal, anterior mediastinum, pineal gland, retroperitoneum, neck, and stomach), frequently elaborate serum markers (AFP and human chorionic gonadotropin), and with application of multimodal therapy, have a favorable outcome.[97] Germ cell tumors take their origin from the fetal yolk sac, and the produced lineage migrates to either the gonad or to the extragonadal sites noted. Teratomas, the most common germ cell tumor, contain all three embryonic layers; ectoderm, endoderm, and mesoderm. Though uncommon in the testes, teratomas are the most common ovarian germ cell tumor, and they are typically most prevalent in extragonadal sites, particularly the sacrococcygeal area. Teratomas are classified as mature or immature, the latter being further graded I to III based on the density of primitive neuroepithelium.

Testicular germ cell tumors in prepubertal children are in aggregate infrequent, with the endodermal sinus tumors being the most common subtype. [97] Seminomas are typically more common in young adults, occurring both in normally descended testes or in undescended testes at an even higher incidence (ten to 30 times greater incidence).[98]

Mature teratomas are the most common ovarian germ cell tumor, and they are benign. In rare circumstances, peritoneal and omental implantation with mature glial tissue occurs (i.e., gliomatosis peritonei), but this is also a benign finding. [99] The most common malignant ovarian germ cell tumor is the AFP-secreting endodermal sinus tumor. Dysgerminomas, like their male counterpart seminomas, are more common in adolescents and young adults. Embryonal carcinomas are the least differentiated of the ovarian germ cell tumors. Choriocarcinoma and mixed tumors are even less common. Patients with a variety of intersex anomalies are at increased risk for developing a gonadoblastoma, a tumor capable of malignant transformation, and such Y-chromosome–bearing gonads are best removed to prevent such transformation, which increases in incidence with age.[100]

The extragonadal germ cell tumors account for almost two-thirds of pediatric germ cell tumors. Such teratomas vary from mature to various grades of immaturity, and transformation to frank malignancy is age related.[101]

The method of operative therapy for a solid testicular tumor requires a transinguinal approach and initial atraumatic occlusion of the cord structures. On delivery and inspection of the testis, enucleation of the mass is acceptable if a teratoma is suspected and proven by frozen section. In contrast, a radical orchiectomy would be done with ligation of spermatic cord structures at the internal inguinal ring along with subsequent retroperitoneal lymph node resection for a malignant testicular tumor. Chemotherapy would accompany all radical procedures for control of stage II, III, and IV tumors.

The standard operative approach for ovarian tumors is not dissimilar (i.e., gonadal sparing for teratomas and a progressively more aggressive approach for malignant tumors).[97] In the latter instance, the operation typically includes aspiration of ascites or peritoneal washings for cytology, peritoneal surface inspection and sampling for suspicious lesions, unilateral salpingo-oophorectomy, contralateral ovarian biopsy if suspicious (bilaterality most common for dysgerminoma followed by teratoma), omentectomy, and retroperitoneal lymph node sampling. Again, adjuvant chemotherapy is the rule for malignant tumors; its role in immature teratomas is less certain.[102]

The main operative challenge in extragonadal teratomas is dictated as much by their size and location as by their likelihood of associated malignancy. The sacrococcygeal teratomas may have a profound hemodynamic influence on the fetus, which may produce fetal hydrops and death. [103] *In utero* tumor ligation or ablation may be appropriate to prevent this outcome, whereas delay of delivery, if possible, to allow for further fetal development is best. [104] At resection, control of the blood supply via the middle sacral artery is most important to minimize intraoperative hemorrhage, but morbidity related to size and location (e.g., neurogenic bladder and rectal compression) remains a significant issue that likely cannot be prevented. [105]

Mediastinal germ cell tumors are typically mature teratomas, and they are best treated by resection. Rarely, when presenting in adolescence, such tumors may be malignant. The biggest operative challenge posed by anterior mediastinal tumors is their compression of either the trachea or superior vena cava.

Cervicofacial teratomas are very rare, but if sizable and located in a site that compresses the airway *in utero*, perinatal management becomes critical. In this scenario, the safe application of *ex utero* intrapartum treatment (EXIT procedure) can safely be applied, assure a secure airway, and permit safe neonatal excision. [106]

**Lymphoma**

The role of the surgeon in Hodgkin's disease and lymphoma is in most cases to establish the diagnosis by biopsy. In the past, staging laparotomy in which the spleen was removed, the liver was biopsied, and multiple abdominal lymph nodes were sampled was frequently used for children with Hodgkin's disease to provide the pathologic stage. One series showed that even with current imaging techniques, a change between the clinical and the pathologic stage occurred in 25% of children and adolescents in whom a staging laparotomy was performed.[107] Nonetheless, current Hodgkin's disease protocols are based primarily on clinical staging guidelines. It must be accepted that a significant percentage of children will be understaged using clinical staging, but with improved systemic chemotherapy and the rare use of radiotherapy as a single modality, this seems to be the sole disease in which accurate staging is now considered less important. It does make it exceedingly difficult, however, to compare series in which pathologic staging has been performed with those with clinical staging alone.

In the NHLs, surgery is also important for diagnosis. In the past, it had been claimed that resection of the primary tumor might improve the long-term survival of children with Burkitt's lymphoma. [108] Recent single and multiinstitutional studies, however, have refuted this claim. [109,110] Extensive surgery often results in surgical complications that delay the onset of treatment and often allow the regrowth of these rapidly dividing tumors. Anesthetic considerations for biopsy of children with mediastinal involvement of Hodgkin's or NHL are discussed in the section Evaluation of Anesthetic Risk of an Anterior Mediastinal Mass.

### Metastatic Disease

In selected pediatric cancers, surgery may be useful for the treatment of metastatic disease. If the primary tumor has been eradicated and no other sites of metastatic disease are evident, pulmonary metastases are excised in children with osteogenic sarcoma. A more favorable prognosis is present in patients with fewer than four pulmonary nodules and a complete resection of all pulmonary metastases. [111] As a practical point, the number of pulmonary metastases found on palpation at surgery often exceeds the number present on CT scan. The overall disease-free survival appears to be approximately 40% for patients with metachronous osteogenic sarcoma metastases.[112,113] The excision of other selected metastatic sarcomas has not been found to be nearly as beneficial, and the indications for surgery are far less clear. Wilms' tumor pulmonary metastases rarely require surgical excision, as there is no apparent survival advantage to surgical metastasis removal compared to the results of chemotherapy and radiation therapy alone. [114]

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# 13

# GENERAL PRINCIPLES OF RADIATION ONCOLOGY

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## INTRODUCTION

The goal of cancer therapy is to free the patient of local, regional, and distant disease. Radiation therapy is a highly effective treatment modality for many pediatric malignancies. An important advantage of radiation is that major vessels, nerves, connective tissues, and hollow viscera can be included in the treatment volume with relatively low risk of producing complications, thus improving cosmetic and functional outcome. However, in pediatric patients the use of radiation therapy demands special attention to both the overall benefits and indications for the use of this treatment and the potential long-term toxicities peculiar to the growing child. [1]

The optimal use of radiation, as a sole treatment modality or as a part of a multi-modality program, requires an understanding of the action of radiation at the physical, molecular, cellular, tissue, and organ level. In general, there are two strategies to enhance the efficacy of radiation therapy. These include (a) improving the physical dose distribution so as to increase dose in the tumor relative to normal tissues and (b) increasing the differential response to radiation between tumors and normal tissues. The former strategy relates to physical parameters, whereas the latter pertains to biologic considerations.

## BASIC PRINCIPLES OF RADIATION THERAPY

### Physical Considerations

Modern clinical radiation therapy uses external radiation beam delivery techniques with x-ray (photon) or electron fields. Beam energies range from 4 to 25 MeV and are produced using clinical linear accelerators. Clinical linear accelerators accelerate electrons to the desired energy. These electrons are than scattered over a broad area to produce a clinically useful electron field. The clinical electron field contains electrons of a near-uniform energy equal to the acceleration energy. This energy determines the penetration depth of the field into the patient, with higher energies penetrating more deeply into the patient. Alternatively, to produce an x-ray field, the electrons strike a high-density target (usually tungsten), resulting in the production of photons. These photons then pass through a flattening filter to produce a clinically useful x-ray field. The x-ray field contains photons of varying energies up to the peak energy of the accelerated electrons. It is customary to denote the energy of an electron field in MeV and to denote the energy of an x-ray field in mV, because of the near-uniform energy of the electron fields and the varying energy of the x-ray fields. High energy x-ray fields (greater than 10 mV) produced by modern high-energy linear accelerators offer the advantage of deeper dose penetration and lower skin doses, compared to old lower energy equipment such as cobalt-60 units. The higher energies are of clear advantage in the treatment of deeply seated tumors, especially those in the thorax, abdomen, and pelvis. In contrast, cranial lesions are often better treated with lower energy x-ray beams (approximately 6 mV) to ensure coverage to shallow lesions and the meninges.

Both x-ray and electron fields interact inside the patient by an ionization process, in which electrons from the atomic shells are released into the cellular environment. These released electrons interact with the cellular molecules, including the DNA molecules, which results in the disruption of the cell's operation. X-rays and electrons, as well as gamma rays emitted by the nuclear decay of radioactive elements, are all forms of ionizing radiation. The energy released in the ionization process relates to the dose deposited in the patient. Radiation absorbed dose is defined as energy, in joules, deposited per unit mass, in kg, and has the unit of J per kg denoted as gray (Gy) in the international system of units (SI units). The gray is named in honor of L. H. Gray, the noted British radiobiologist, and replaces the older unit of rad, where 1 Gy equals 100 rads.

A clinical linear accelerator has many degrees of geometric movement and can position a treatment field at almost any orientation relative to the patient. This is achieved by mounting the radiation production source on a gantry that fully rotates around the patient. In addition, the patient is positioned on a treatment couch that permits the patient to pivot with respect to the gantry rotation plane. This degree of movement is necessary both to permit an optimal approach to the target volume and to permit multiple fields to approach the target volume. The radiation field can be shaped to geometrically conform the target volume intersection with the field by a collimator assembly mounted on the gantry. The field shape can be adjusted by inserting manually produced apertures that block the radiation in selected sections of the radiation field or by adjusting the settings of a multi-leaf collimator, which is a device that has a large number of opposing vanes, or leafs, that span the field. The adjustment of individual leafs permits the multi-leaf collimator to assume a large variety of radiation-blocking patterns. The multi-leaf collimator is the key to the latest development in delivery technology that permits the radiation field itself to be spatially modulated in a technique called *intensity modulated radiation therapy* (IMRT) in contrast to the “conventional” radiation fields that have a constant, or fixed, intensity profile across the field area. It is expected that IMRT will have a significant clinical impact.

A patient treatment, in general, involves multiple nonoverlapping treatment fields, each of which enters the patient and focuses on the target volume containing the tissues to be treated. The set of multiple fields reduces the dose to non-involved tissues by, in effect, spreading the dose outside the target volume over a much larger volume. Sophisticated treatment field configurations, or treatment plans, are designed with computer-based techniques using patient-specific computed tomography (CT) and magnetic resonance imaging (MRI) information in combination with models for managing the treatment field geometry with respect to the patient anatomy and models for computing the dose inside the patient. Such treatment planning techniques result in dose distributions that can precisely conform to the target volume. These important technical advances are discussed in the section on Recent Advances in Radiation Therapy.

In addition to conventional external beam irradiation, other specialized modalities are available for the treatment of some malignancies. One such technique is the brachytherapy implant technique. Brachytherapy places one or more radioactive sources into a body cavity (i.e., intracavitary implants), such as the vagina, or directly into the tumor (i.e., interstitial implants). For permanent implants, radioactive sources are placed into tumors under ultrasound, CT, or MRI guidance. For temporary implants, hollow catheters or other loading devices are positioned at the time of surgery, and remotely loaded, and emptied, with radioactive sources. Both techniques allow for high doses of radiation to be delivered to relatively small volumes of tissue. Brachytherapy techniques can deliver high doses to target tissues, with excellent sparing of nearby healthy tissues. Iridium-192 and iodine-125 are the most frequently used radionuclides. Cesium-137 has been largely replaced in favor of iridium-192, with the availability and popularity of automated remote afterloading equipment. Radionuclides are embedded in seeds and sealed in thin plastic strands that can be inserted into implanted catheters. Iodine-125 has a short half-life of 57 days and is often used as a permanent implant, especially in prostate and central nervous system treatments. Iridium-192 has a half-life of 70 days and permits reuse of the seeds in some case, notably when used with the automated remote afterloaders.

Brachytherapy treatments and external beam treatments differ in radiobiology. [2] Implanted sources deliver continuous doses of 30 to 100 cGy per hour over the duration of the implant. The target volume is thus treated continuously with a low dose rate over a long period. This is in contrast to an external beam treatment, in

which the target volume receives a short pulse of dose (on the order of 100 to 200 cGy) once or twice a day over many (5 to 7) weeks. The different dose rates and delivery patterns affect cell kill for a given total dose because of a difference in repair of sublethal damage. Thus, using a low dose rate may result in less damage to normal tissue.[2]

Brachytherapy in the form of intracavitary or interstitial implants has been used for some pediatric malignancies. For example, brachytherapy has been used in rhabdomyosarcomas of the genitourinary tract as well as in retinoblastoma[3] and central nervous system tumors in an attempt to limit the dose to surrounding normal tissues.[4,5,6,7] and [8]

**Biologic Considerations**

Cells vary considerably in their radiosensitivity depending on their position in the cycle. Cells in mitosis (M) and early in the DNA synthesis phase (S) have the greatest sensitivity whereas cells in late S phase as well as in $G_2$ have the least sensitivity.[2] The target for radiation-induced damage in tumor cells is the DNA. Unrepaired or misrepaired double strand breaks presumably lead to cell killing although several factors modify the response of tumor cells and normal tissues to radiation. These include: (a) the inherent radiosensitivity of the cells in question (b) the capacity of these cells to repair radiation damage, (c) the oxygen and nutrient status of the tumor, (d) the position of an individual cell in the cell cycle, and (e) the capacity for repopulation. To improve the therapeutic differential between tumor cells and normal tissues, radiation treatments are given as a series of equal-sized fractions over a number of weeks, in part exploiting these factors.

The basis for the exquisite sensitivity of certain tumor cells to radiation, such as seminoma, in contrast to relatively radioresistant tumors, such as osteogenic sarcoma, is poorly understood. Genetic factors may play a role in determining the response of cells to radiation. There has been a wider recognition of the process of programmed cell death or apoptosis. Apoptosis appears to be one mechanism for interphase death at very low doses of irradiation.[2] This process of cell death is not linked to mitosis, was first appreciated in lymphocytes, and is known to occur to varying degrees in normal and tumor tissues. Of interest, the normal p53 gene has been shown to be required for this process.[9] Tumor cells lacking a normal p53 gene may therefore be more resistant to cell killing if this process is turned off.

In general, both normal tissues and tumor cells are capable of repair of a portion of the radiation damage between doses of radiation. Normal tissues may possess greater capacity for repair although they may require more time to do so. Therefore, spacing radiation fractions by at least 6 hours (usually 24 hours) may give a greater advantage to normal tissues. Even small therapeutic gains, when amplified exponentially over a course of treatment, can become very significant. Molecular oxygen is a potent radiosensitizer, and therefore tumor cells at low oxygen tensions are relatively resistant to radiation. This situation can occur because a fraction of tumor cells are positioned progressively further from the capillaries. With tumor growth, they can outgrow their blood supplies. As cells closer to the capillaries are most sensitive, they will be preferentially killed. Thus, there are fewer cells metabolizing oxygen, and those that remain will be “re-oxygenated” and more sensitive. Therefore, fractionated radiotherapy would be beneficial in providing the opportunity for a net improvement in oxygenation and hence tumor cell sensitivity.

The phenomenon of repopulation occurs in acutely responding normal tissue, such as the gut epithelium, but also occurs in tumors. A therapeutic advantage would occur if the rate of repopulation was more rapid in normal tissues than in tumors. In general, it is best to deliver a course of radiation in the shortest period that will be tolerated by acutely responding normal tissues. Delays in treatment beyond this time, due to radiotherapy toxicity or elective breaks, may adversely affect prognosis.[10]

***Radiation Interactions with Chemotherapeutic Agents***

A number of chemical substances will alter the radiation response of a cell.[11,12,13,14,15] and [16] The effect may be additive, such as with most alkylating agents and antimetabolites, or it may be synergistic, such as with the antibiotic dactinomycin.[14] Doxorubicin and dactinomycin markedly reduce the “shoulder” region of the radiation cell survival curve.[14,15,16,17] and [18] Dactinomycin also steepens the slope of the exponential portion of the cell survival curve and potentiates the radiation effect; thus, it is thought to be a true radiation sensitizer.[14]

An interesting clinical phenomenon known as “recall” has been demonstrated with combinations of radiation and either doxorubicin or dactinomycin. Even with time intervals as long as 3 weeks between the use of radiation and dactinomycin, a brisk reaction can be seen with striking recurrence of the prior radiation reaction.[13] Such “recall” reactions are usually used to describe skin erythema that returns with each subsequent dose of chemotherapy. Radiation may also alter drug metabolism, thereby increasing the combined toxicity.[16] This has been shown in children with right-sided Wilms' tumor, in which hepatic irradiation combined with dactinomycin resulted in veno-occlusive disease, although veno-occlusive disease is also seen without radiation therapy at a 2% incidence. The incidence in the National Wilms' Tumor Study-4 for both the standard regimen of dactinomycin and the pulse regimen was 10 times more frequent than that in the National Wilms' Tumor Study-3 despite no change in the radiation therapy.[17] In addition, the neurotoxicity of vincristine is enhanced, presumably because of delayed metabolism of the drug by the liver.

Etoposide (VP-16) inhibits cell replication. Sublethal lesions produced by radiation become lethal lesions with etoposide, resulting in increased cell killing when this agent is used with radiation therapy. In addition, cells that are arrested in $G_2$ by irradiation are particularly sensitive to etoposide.[18]

Among other chemotherapy agents frequently used in pediatric malignancies which interact with radiation are the antimetabolites. Methotrexate (MTX) is the most widely used clinically. It is a cycle-specific agent, altering the cell kinetics of the surviving cells. Cells in mitosis are more sensitive to irradiation. This leads to an enhanced response to the combination of MTX and irradiation. MTX selectively kills cells in S phase, leaving a greater proportion of cells in G1. As the surviving cells proceed through the cell cycle, they are differentially more or less sensitive to subsequent irradiation. MTX also inhibits repair of DNA strand breaks, which acts to enhance the toxicity of irradiation. The combination of cranial irradiation followed by systemic administration of MTX can produce a subacute syndrome known as *necrotizing leukoencephalopathy*, characterized by lethargy, seizures, and cognitive and cerebellar dysfunction.[19,20] Careful attention to the sequencing of MTX and cranial irradiation as well as the dose and route of administration (i.e., intrathecal versus intravenous) can minimize the likelihood of this devastating toxicity.

Paclitaxel (Taxol) blocks progression of the cell into mitosis. This drug blocks cells in $G_2$ per M by inhibiting depolymerization of tubulin.[21] *In vitro* studies have shown a significant radiosensitization with paclitaxel, even at less than cytotoxic doses.[22] Several clinical trials are ongoing to assess the combined use of paclitaxel and MTX as radiosensitizers.

***Dose Rate and Fractionation Effects***

The dose rate (Gy per minute) as well as the fraction size are major factors in determining the biologic effect of a given absorbed dose of radiation.[2] As the dose rate is reduced, the slope of the survival curve becomes less steep while the extrapolation number tends toward unity. This dose rate effect may be primarily due to repair of sublethal damage and is most dramatic between 1 and 200 cGy per minute. There is tremendous variation in the magnitude of the dose rate effect. For example, normal bone marrow stem cells generally demonstrate little dose rate effect and are characterized by a small shoulder (i.e., little repair capacity).[23,24] This is in contrast to the gastrointestinal tract, which has a broad shoulder and a correspondingly large dose rate effect.[2] It is this differential sensitivity in repair capacity between gastrointestinal tract and bone marrow stem cells that permits a differential effect of total body irradiation for bone marrow transplantation.[2,26] Pulmonary alveolar cells also demonstrate a significant repair capacity, and the incidence of pneumonitis at whole body doses of 9 to 10 Gy decreases significantly as the dose rate decreases to 2 to 5 cGy per minute.[25] One example of the importance of fractionation in clinical practice was shown with the use of total body irradiation for bone marrow transplantation. Cyclophosphamide along with single fraction total body irradiation of 9 to 10 cGy delivered at approximately 5 cGy per minute resulted in some long term remissions; however, most patients died of complications or recurrence of leukemia.[26] A prospective randomized clinical study comparing single-dose total body irradiation to a fractionated schedule of daily radiation was initiated.[27] Patients with acute nonlymphoblastic leukemia were randomized to receive either 10 Gy in a single fraction in 1 day or 12 Gy given in 2 Gy fractions over 6 days. Survival was significantly better in the fractionated group. The incidence of leukemic deaths was identical; the improved survival was due to a decrease in deaths from complications. This study demonstrated that 12 Gy given over 6 days was equivalent to 10 Gy in a single dose for leukemic cell kill, but allowed greater repair of dose-limiting normal tissues such as the lung. Various combinations of fractionated and low-dose rate techniques have become widespread.[26,27,28] and [29] Whereas the optimal schedule is still uncertain, it is clear that fractionation spares normal tissue toxicity to a greater extent than single-fraction total body irradiation. Low dose rate allows repair of normal tissues; however, the use of fractionation appears to allow greater repair and to decrease the late effects over that achieved with low-dose-rate, single-fraction radiation.[30,31,32,33] and [34]

*Fractionation* refers to the amount of radiation given at each session (i.e., fraction size) and the overall number of treatments ( Table 13-1). *Hyperfractionation* refers to the use of a larger number of fractions with smaller than conventional doses per fraction over the same treatment time.[31] The use of standard fraction sizes given

more than once a day is referred to as *accelerated fractionation*. Theoretically, hyperfractionation will result in an improvement in the therapeutic ratio when late normal tissue reactions are dose-limiting.[31] Clinically, hyperfractionation and accelerated fractionation schedules are being used in most total body irradiation regimens to decrease the overall time of irradiation (i.e., number of days) while giving the same or greater total dose.

| Conventional fractionation | Once-daily treatment with radiation usually given in 1.5–2.0 Gy/fraction per day |
|---|---|
| Hyperfractionation | Total dose is increased, size of dose per fraction is reduced. Overall treatment time is relatively unchanged. Usually given as twice-a-day treatment. |
| Accelerated fractionation | Overall time is reduced, the dose per fraction is unchanged or somewhat reduced. |
| Accelerated hyperfractionation | Incorporates features of both accelerated and hyperfractionation. |

From Denekamp J. Repair rates: from basic studies to the clinic. Radiother Oncol 1989;14:303–305.

**TABLE 13-1. RADIATION FRACTIONATION SCHEDULES**

Laboratory evidence supports the potential benefit of hyperfractionation in the pediatric population.[33,34] The clinical trials to date are limited, and so far, have not shown a benefit with respect to improved tumor control.[35,36,37 and 38]

### Clinical Considerations

Radiation is a highly active antitumor agent. Virtually all tumors respond favorably to irradiation, and a significant number are permanently controlled. However, the rate of response is not always predictable. For many tumors, response can be seen over years.[39] The criteria such as “complete response” or “partial response” have limited value, and permanent or long-term, local control relapse-free survival and overall survival are usually a better gauge of treatment efficacy.

The general relationship between dose and the probability of curing a tumor is shown in Figure 13-1. The delivery of the physical dose to the tumor is controlled by the physical factors discussed under Physical Considerations, whereas the shape of the curve is determined by the biologic factors. Normal tissues also display similar sigmoidal dose response curves, although fractionated therapy generally places this curve to the right of the tumor curve. At doses that produce no level of complications, cure rates are low, whereas tumor doses that produce greater than 90% cure rates may produce unacceptable morbidity. In general, intermediate doses are chosen that balance benefits and risks. Of note, the addition of chemotherapy may markedly shift these curves. As a guiding principle, it is often the balance of these potential risks and benefits, the therapeutic ratio, that determines the doses given for a particular patient.

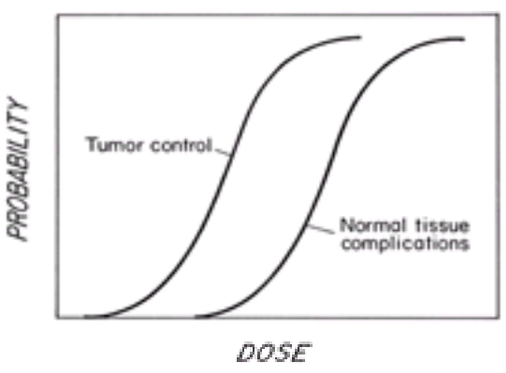


**FIGURE 13-1.** Idealized sigmoid curves depicting the probability of tumor control compared with the probability of normal tissue complications. The therapeutic ratio refers to the balance between these two curves. In general, as the dose increases, the risk of complications increases.

The optimal balance between control and complications varies in different clinical settings depending on the consequences of local relapse or the severity of the complications engendered. When the complication results in severe morbidity (e.g., radiation myelitis), then it must be avoided even if the price is an increased rate of tumor relapse. However, if patients who develop local tumor failure after irradiation cannot be salvaged and normal tissue complications can be managed by medical or surgical means (e.g., small bowel obstruction), then a significant rate of complications may be a reasonable price for a higher proportion of cured patients. For example, in pediatric Hodgkin's lymphoma, irradiation of smaller volumes with planned chemotherapy produces an equivalent rate of tumor control with less morbidity than the previous use of wide-field irradiation.[40] In early-stage Hodgkin's disease, irradiation alone results in an excellent disease-free survival.[41] However, in the pediatric age group this decision is complicated by the direct effect of radiation therapy on bone growth.[42,43] Thus, in patients with early-stage disease with significant growth remaining (those younger than 14 years of age), chemotherapy, usually with involved field, low-dose radiation therapy, is the standard of care.[44,45,46,47 and 48]

Parameters that can be varied during a course of treatment include the volume irradiated, the total dose, the fraction size, and the dose rate used at each treatment session. Also, the time interval between each radiation fraction and the overall time between initiation and completion of radiation can be varied.[49,50] Each of these features has significant bearing on both tumor control probability and normal tissue complications. In general, as treatment volumes increase, the total dose must decrease to maintain a given level of complications. Similarly, an increase in radiation fraction size also results in an increase in late complications of the total dose, or volume is not reduced.[51,52]

The total dose required for tumor control is largely dependent on the number of tumor cells that are present and the specific tumor in question. Table 13-2 shows some examples of the total dose used for various diseases. Most hematologic malignancies are radiosensitive and require only a moderate total dose for local control. For example, doses of 36 Gy provide excellent local control in Hodgkin's disease when radiation alone is used, whereas doses of greater than 50 Gy are required for soft tissue sarcomas. Generally, these principles of total dose and radiation schedule are not age related. No data are available to justify a variation in radiation dose based on age. The one exception appears to be neuroblastoma in the child younger than 12 months old, in which the biology of the disease seems to be different than in older children. Modifications of total dose may, however, be justified by age-related toxicity. For example, dose reductions in the treatment of brain tumors in infants have been recommended. This is because myelinization is incomplete, and the functional impairment in this age group is of great concern. In this case, lower control rates may be justified by the decrease in complications.

| Tumor | Radiation dose (Gy) |
|---|---|
| Hodgkin's disease | |
| Radiation therapy alone | 36–40 |
| Combined modality | 15–25 |
| Rhabdomyosarcoma | 40–55 |
| Neuroblastoma | |
| <12 mo | 15 |
| >12 mo | 20–35 |
| Histiocytosis | 4.5–10.0 |
| Acute lymphoblastic leukemia (central nervous system treatment) | 18–24 |
| Medulloblastoma | |
| Craniospinal | 23–40 |
| Posterior fossa | 54–55 |

**TABLE 13-2. GENERAL RADIATION DOSES FOR SPECIFIC TUMORS**

## RECENT ADVANCES IN RADIATION THERAPY

A modern linear accelerator is mechanically accurate and offers precise control of the delivery of the radiation dose to the patient. An early high-precision application of the linear accelerator was in small-field, high-precision stereotactic therapy. Stereotactic therapy was first practiced by proton and gamma knife techniques. [53] The concomitant availability of sufficiently powerful computers and the development of image-based radiation therapy treatment planning software permitted stereotactic therapy to move from a special-purpose technique only available at selected centers to the radiotherapy community at large. Stereotactic therapy uses a large number of narrow beams of radiation delivered as single-fraction stereotactic radiosurgery or as multiple-fraction stereotactic radiotherapy. The large number of narrow fields permit an exquisite focus of radiation dose on the target volume with little dose to the healthy tissues. (This can be compared to, by analogy, the effect of focusing sun rays through a magnifying glass, in which the focus point intensity is much higher compared to any other point.) The large number of beams is typically delivered by continuously rotating the radiation source, located in the gantry/collimator assembly of the linear accelerator, across the patient. This rotation movement is a basic parameter of the linear accelerator and is achieved by rotating the gantry over multiple intervals at multiple positions of the patient treatment couch. A fundamental feature of stereotactic therapy is a stereotactic frame that attaches rigidly to the patient. This frame is surgically attached to the cranium for single-fraction, single-day treatment, radiosurgery. Stereotactic radiotherapy, in contrast, requires that the patient return for many consecutive days and relies on a relocatable frame that typically uses the patient's upper dentition to achieve rigid positioning and accurate reproducibility. The stereotactic frame forms the basis for an absolute reference system in which all geometric parameters, such as patient setup and beam approach, are defined. The patient is imaged with the frame and a fiducial (marker) reference system. This image procedure permits a volumetric image reconstruction based on the fiducials with a precision above and beyond the precision of the CT or MRI scanner. Stereotactic therapy provides a complete operational model for high-precision treatment delivery and defines procedures for patient imaging, volumetric reconstruction of the patient's anatomy, modeling of the required radiation fields to satisfy the clinical prescription, and the delivery of the treatment.

Arteriovenous malformations were the original indications for stereotactic radiosurgery, [54] as these could be located with angiographic procedures and did not require CT imaging for treatment definition. The availability of CT imaging dramatically expanded the applicability of stereotactic radiosurgery. Currently, the most common indication is in adult patients with metastatic disease to the brain. In pediatric patients, in whom metastases to the brain are rare, single-fraction stereotactic radiosurgery is largely used as a “boost” after conventional fractionated radiation therapy or for recurrent brain tumors that have been previously treated with conventional radiation therapy. [55,56 and 57]

Multiple-fraction stereotactic radiotherapy, in contrast to the large single fraction of stereotactic radiosurgery, delivers corresponding focal doses but with conventional fractionation and the use of a noninvasive frame to reproduce accurate patient setup over the course of several weeks of treatment. [58] The patient shown in Figure 13-2 is a 12-year-old girl with a symptomatic low-grade astrocytoma. This figure shows the dose distribution and beam arrangement for a stereotactic plan. This dose distribution, for the same patient as in Figure 13-2, was obtained by applying five arc rotation segments (Fig. 13-3), in which each arc segment had a collimated shape determined by a circular aperture trimmed by the linear accelerator collimator jaws. The treatment plan incorporated CT imaging under stereotactic guidance and incorporated MRI for accurate definition of the low-grade neoplasm. The MRI was obtained without stereotactic localization and was fused to the CT image data using an automated fusion technique. [59,60 and 61] All stereotactic technology produces highly focal dose distributions, making the treatment ideal for treating localized lesions that are not widely infiltrative. Stereotactic technology is most often applied to tumors of the brain, as the cranium affords excellent immobilization and presents no internal organ motion. More recently, stereotactic treatment principles have been applied to thoracic and abdominal sites. [62] Tumors well suited for treatment with stereotactic radiotherapy are small (generally 5 cm or less), noninvasive, radiographically distinct, and known to be well controlled with conventional radiation therapy. [62]

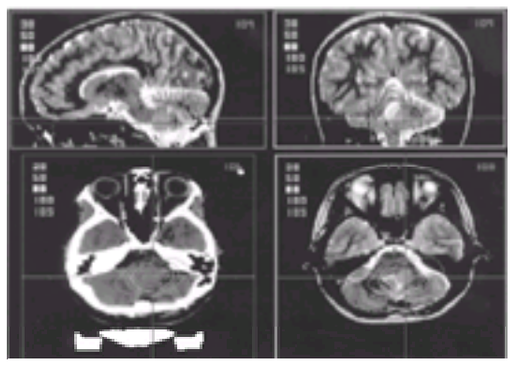

**FIGURE 13-2.** The patient is a 12-year-old girl with a low-grade astrocytoma. The dose distribution is shown superimposed on the sagittal, coronal, and transverse magnetic resonance (MR) sections through the center of the radiation field (radiation isocenter). The corresponding transverse computed tomography (CT) slice is also shown (*lower left*) in contrast to the soft tissue contrast of MR (*lower right*). Radiation treatment planning relies on both CT and MR modalities. The CT is required to accurately calculate the radiation inside the patient, whereas the MR in this case is required to reconstruct the target volume and the nearby brainstem. Dose lines are shown in percent of dose prescription of 50.4 Gy delivered in 25 fractions of 1.8 Gy. (See Color Figure 13-2.)

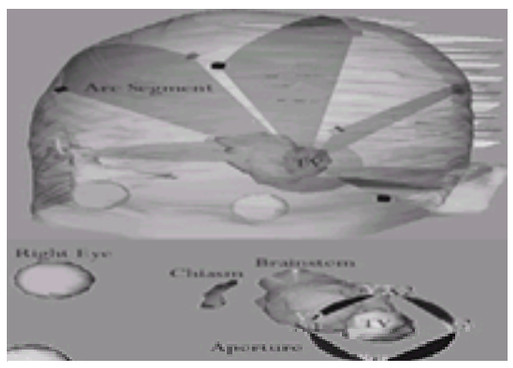


**FIGURE 13-3.** The three-dimensional reconstruction of the same patient as in Figure 13-2. The patient's external surface together with the eye globes, the brainstem, and the target volume (TV) as shown in the top part. The patient is treated with five arc segments of radiation therapy indicated by the pie-shaped wedges. The shape denotes the traversal of the central axis of the radiation field through the patient's anatomy. All five axes intersect at the radiation isocenter inside the target volume. The bottom part illustrates (for one angle for one arc segment) the view obtained by placing the observer's eye at the radiation source and showing the relationship of all organs and the radiation field. The radiation field is emitted only through the opening formed by the circular aperture and the four standard accelerator collimator jaws (labeled X1, X2, Y1, and Y2). The target volume is clearly included in the opening and thus receives full dose from this approach. Some brainstem is unavoidably involved but is kept below the tolerance dose for radiation complications. This view is called the “beam's eye view” and is checked for all radiation approaches to obtain a minimal shape for each arc segment.

The goals of stereotactic therapy are a high rate of tumor control and minimization of normal tissue exposure, goals that are ideally suited for treatment of pediatric patients. [56,57,63,64,65 and 66]

**Intensity Modulated Radiation Therapy**

A fundamental development in radiation oncology is the recent introduction of IMRT. IMRT uses many radiation fields, more than conventional focal radiotherapy, and each field has a unique radiation intensity profile that varies arbitrarily as a function of position within the field. This is in sharp contrast to conventional radiotherapy in which each field has either a uniform intensity or a standard, predetermined, intensity profile. IMRT delivery, in general, uses the multi-leaf collimator to vary the intensity locally during the delivery of the field. This is achieved by precisely controlling both the shape of open regions within the field and the amount of radiation, or intensity, that is emitted in those regions. The combination of geometric control and radiation delivery can create an intensity profile over the field of almost arbitrary shape.

The ability to control the intensity over the field area dramatically increases the number of treatment parameters that control the actual dose delivered in the patient. A conventional treatment field typically has four parameters for the treatment. In contrast, an IMRT field, with its fine control over the intensity distribution, has on the order of 100 to 1,000 parameters, in which each parameter represents a local value of the intensity inside the radiation field. This number is beyond the capability of a human treatment planner and can only be determined through the use of computer-based algorithms. These algorithms take as primary input the geometric shape and location of the target volume and other dose-critical structures together with the dose parameters, such as maximum and minimum dose, for each structure. In addition, the algorithm takes the set of field approaches to expedite the execution of the algorithm. In fact, IMRT solutions are somewhat dependent on the choice of treatment field approaches. The algorithm then manipulates the set of intensity values for all fields until the desired dose of radiation to the target volume is achieved, whereas the dose outside the target volume, and especially any critical structures, is minimized. The set of intensity values is then converted to the set of parameters required to control the multi-leaf collimator that delivers the IMRT fields.

IMRT treatments require much of the same delivery framework as used in stereotactic radiotherapy (i.e., precise imaging and accurate, reproducible setup). This is a complicated task, as IMRT is used in noncranial sites where internal organ motion and patient fixation are major concerns. A prime example of the use of IMRT is the dose escalation study at Memorial Sloan-Kettering Cancer Center, which used 81 Gy in prostate cancer patients to improve control rates. [67] Such high dose levels would not be achievable using standard radiation techniques. IMRT treatments are effective in reducing dose to a critical structure, especially when the critical structure indents the target volume (Fig. 13-4). The ability to create concave dose distributions is perhaps the most dramatic feature of IMRT. IMRT can also be effective in reducing dose to normal tissues. There is, however, a relationship between the target volume size, the resolution of the multi-leaf collimator, and the achievable reduction to healthy tissues. A typical multi-leaf collimator has a resolution of 1 cm (i.e., the intensity can be controlled within an area of 1 cm by 1 cm) and the whole radiation field is subdivided into subareas of 1 cm by 1 cm, in which each subarea can deliver a different intensity to control the shape of the delivered dose distribution in the patient. A typical conventional target volume is on the order of $10 \times 10 \times 10$ cm $^3$ and the number of subareas typically is on the order of 100 per field. A typical IMRT treatment involves on the order of seven fields, and an optimization algorithm therefore must compute on the order of 1,000 intensity values for a treatment. If, however, a target volume is “small,” on the order of 3 to 5 cm, the number of 1 cm by 1 cm subareas per field that irradiate the volume can be as small as 10, and the number of intensity values is on the order of 100. This is a dramatic reduction in the number of parameters that the algorithm can control to achieve an optimal solution and can reduce the effectiveness of IMRT compared to conventional focal treatment. Conversely, if the target volume is too “large,” reduction in dose to healthy tissues is compromised simply by geometric constraints, as the ratio of healthy volume to target volume becomes small and dose cannot be effectively distributed over the healthy volume to achieve adequate reduction. These considerations will require considerable study to optimize IMRT delivery for specific treatment sites.

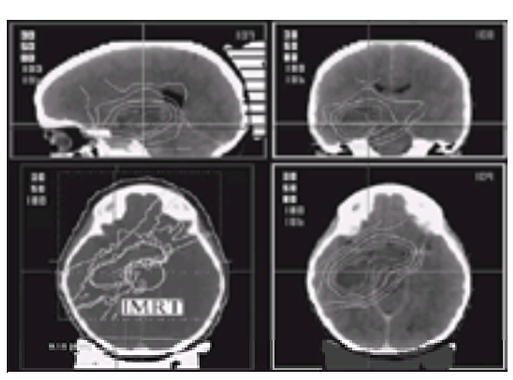


**FIGURE 13-4.** The dose distribution from four static fields is shown for a 16-year-old girl with an optic glioma. The optic glioma is shown as a darkened area on the top and right bottom slices and as a white contour on the intensity modulated radiation therapy (IMRT) slice. The dose distribution for the top and bottom-right panels is obtained by nonintensity modulated fields (conformal radiation). The dose distribution in the IMRT slice uses the same set of four fields but uses an IMRT optimization to obtain field-specific intensity for each of the four fields. Note that the IMRT dose distribution visibly reduces the dose to the brainstem compared to the bottom-right section.

Consider for illustration purposes a 16-year-old girl with an optic glioma. A typical conformal plan might use four static fields and yield the acceptable, but perhaps not optimal, dose distribution shown in Figure 13-4. The optic glioma is highly irregularly shaped, and the four-field dose distribution will, of necessity, involve the brainstem. The bottom left panel in Figure 13-4 uses the same set of four fields, but has used an IMRT optimization to adjust the intensities in each of those four fields. Note that the IMRT optimized dose distribution is able to noticeably spare the brainstem compared to the non-IMRT dose distribution. Beyond the brainstem, however, it is hard to evaluate whether there is an improvement for the IMRT dose distribution. It should be noted that a four-field configuration is a minimal set for IMRT delivery.

The quantification of the appropriate technique, albeit conventional conformal with or without IMRT, requires quantification indices. One such index is the tissue-volume ratio defined by Nedzi et al. [68] as the ratio of the volume of healthy tissue to target volume tissue at the prescription level. It is not clear what the best technique is given the physical presentation of the treatment geometry (notably the size and location of the target volume) and the delivery device. Bues et al. [69] discuss the use of IMRT for small intracranial lesions (approximately 6 cm maximum dimension) and compares the efficacy of three competing techniques: conventional arc delivery described by Hacker et al., [61] IMRT delivered with a 1.0 cm resolution, and IMRT delivered with a 0.5 cm resolution ( Fig. 13-5). Figure 13-6 shows the dose-volume histogram, a graph that indicates how much volume (in cc or percent total volume) receives a given dose or more, for the brainstem volume for these three delivery options. A clear advantage for both IMRT delivery modes is quantified, compared to the qualitative assessment from Figure 13-4. Figure 13-7 shows the dose-volume histogram for other healthy tissue (i.e., excluding the brainstem) for the 1.0-cm and 0.5-cm resolution IMRT modes. The high-resolution IMRT delivery achieves a significant reduction in healthy tissue dose by a factor of 2! To assess the efficacy of a particular treatment delivery mode, it is useful to consider the distribution over a particular patient population as a whole. Figure 13-8 shows the range of tissue-volume ratios achieved with each treatment mode for a patient population presenting with cranial neoplasms having a maximum dimension up to 6 cm. Although for the patient in Figure 13-5 the 1-cm IMRT delivery was clearly superior overall, in general, 1-cm IMRT does not significantly improve the treatment for the population as a whole. In contrast, the 0.5-cm IMRT mode does improve the treatment for all patients.

**FIGURE 13-5.** The intensity maps for a single field for 1-cm resolution intensity modulated radiation therapy (IMRT) **(A)** and 0.5-cm resolution IMRT **(B)** are shown. Note that both fields outline the target volume shape along the beam direction.

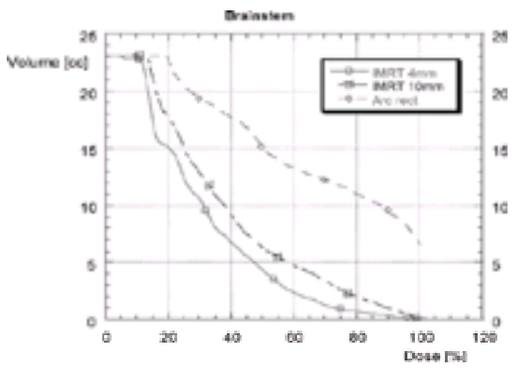


**FIGURE 13-6.** The dose volume histogram shows what volume (in cc or in percent total volume) receives a given dose of radiation. Three treatment delivery scenarios are considered for the patient shown in Figure 13-4: a collimated stereotactic delivery method,[61] conventional intensity modulated radiation therapy (IMRT) at 1.0 cm resolution, and high-resolution IMRT at 0.5 cm resolution. As was visually apparent in Figures 13-4 and 13-5, IMRT can significantly reduce the dose to a critical structure, such as the brainstem adjacent to the target volume in this case.

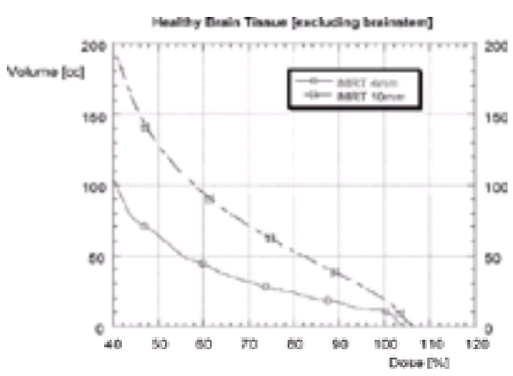


**FIGURE 13-7.** The dose-volume histogram for healthy brain tissue, but excluding the brainstem, indicates a significant reduction (by a factor of 2) for the higher resolution intensity modulated radiation therapy (IMRT) delivery mode.

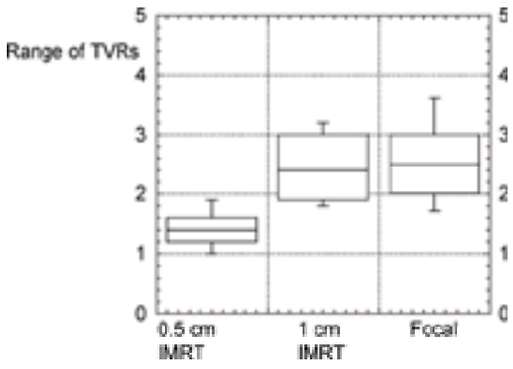


**FIGURE 13-8.** Range of tissue volume ratios (TVRs) across the patient population for each treatment modality shown as boxplots. A boxplot shows the median and the quartile extents. Note the broader variation in TVR values for 1-cm intensity modulated radiation therapy (IMRT) and focal delivery compared to 0.5-cm IMRT.

IMRT, as well as other focal techniques, will play a significant role in the treatment of pediatric tumors, as dose reduction to normal tissues is considered of direct benefit to this population in whom growth is a significant issue.

**Proton Radiation Therapy**

The state-of-the-art technique for achieving precise dose localization in a tumor and reducing normal tissue irradiation is proton radiation therapy. Protons are charged particles with a mass of one. Protons deposit their energy over a short distance known as the *Bragg peak*. Their biologic effectiveness is similar to x-rays, resulting in similar cell killing effects. The advantage of protons lies in this physical dose distribution. Because of the defined range of protons, dose distributions can be designed that conform more closely to a tumor volume. This advantage is illustrated in Figure 13-9. The result is a much greater ability to reduce radiation dose to nontarget normal tissues and allows a greater dose to be delivered to the tumor. Clinical studies of proton beam therapy for cancer patients have been ongoing since 1974 at the Harvard Cyclotron Laboratory in Boston, Massachusetts. Other centers that have subsequently commenced proton therapy for cancer patients include: Loma Linda University, California; PSI near Zurich; Uppsala, Sweden; ITEP, Moscow; Capetown, South Africa; Tsukuba, Japan; and Orsay, France. The proof of principle of proton therapy has been in the treatment of skull base chondrosarcomas and chordomas and uveal melanomas.[70,71] Because of the high capital costs required to establish a proton treatment facility, this will likely remain a specialized type of treatment.

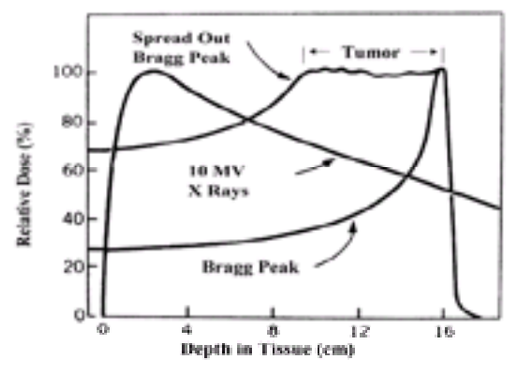


**FIGURE 13-9.** This chart illustrates the differences in protons compared to photons. The curve for the 10 mV x-rays shows more dose deposited before the tumor and continuing dose given to normal tissue beyond the tumor. Note that on the curve labeled “Bragg Peak,” the dose is too narrow to treat the tumor. But the dose shown in the curve labeled “Spread Out Bragg Peak” treats this idealized tumor and has no exit dose beyond the tumor.

The use of protons in pediatric tumors, such as medulloblastoma, is of great interest. [63,72,73] The standard treatment requires the use of craniospinal irradiation followed by a boost to the tumor bed. Acute effects (e.g., nausea and vomiting) as well as late effects to the heart and lungs should be decreased by protons in place of photons for this technique. Figure 13-10 demonstrates the exit dose from conventional photons in contrast to protons. There is no exit dose to the stomach and bowel using protons as compared to 60% of the radiation dose with conventional photons. In addition, there would be no scatter dose to the ovaries in female patients.[74]

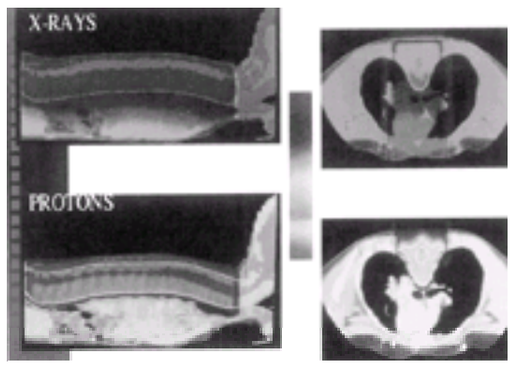


**FIGURE 13-10.** Shown here is the radiation dose in a 43-month-old child with medulloblastoma. The top figures demonstrate the dose from conventional x-rays given to the spine. Because x-rays deposit their energy over a greater distance, 60% of the dose exits to the stomach and intestine. The bottom figures are for protons showing no exit dose to the gastrointestinal tract, lung, or heart. (See Color Figure 13-10.)

### Intraoperative Radiation Therapy

Conventional radiation treatment, delivered to the thoracic, abdominal, and pelvic cavities, is limited by the tolerance of normal tissues such as the heart, lung, stomach, liver, and bowel. For example, it is difficult to deliver doses of greater than 50 Gy to a pelvic tumor because of the risk of injury to the small bowel. However, doses that minimize normal tissue injury are sub-optimal for tumor eradication. One method of escalating radiation dose, beyond that which can be achieved with conventional external beam techniques, is to give the radiation treatment during a surgical procedure. With the tumor bed or gross tumor surgically exposed and normal tissues packed out of the way, a beam of radiation can be delivered during the operation, in the operating room theater. This technique is available at a growing number of institutions.[75,76,77,78,79,80 and 81]

## GENERAL RADIATION THERAPY

Most pediatric malignancies are managed with combined modality therapy. There are only a few tumors in which radiation therapy after surgery is used in the pediatric population. One such example is with some of the nonmalignant brain tumors such as craniopharyngioma and low-grade astrocytomas. [82]

There are also a number of rare benign entities of bone and soft tissue in which radiation therapy has shown efficacy in local control. These include aggressive fibromatosis (desmoid) of soft tissue and giant cell tumors of bone.[83,84] Despite these generally good results, surgery should be considered as the first treatment option for most benign diseases. When surgery is not feasible or if patients have had recurrences after adequate cancer surgery, or if surgery will produce a significant functional decrement, then radiation should be considered.

Radiation has also been used in a number of nontumorous conditions. For example, radiation can prevent coronary artery restenosis after angioplasty. In this application, a temporary dwelling radioactive source is introduced after balloon angioplasty or a permanently-dwelling radioactive stent is placed. [85] In these two instances, the mode of radiation delivery is by brachytherapy. Radiation can also prevent renal allograft rejection [86] and the formation of heterotopic bone formation after total hip arthroplasty.[87]

### Palliative Therapy

Radiation therapy is an effective means of palliating cancer pain that is not responsive to analgesics, narcotics, or other therapies. Patients sometimes notice pain relief after several treatments. The goal of any palliative therapy should be to improve the status of the cancer patient without causing side effects from the treatment. Also, the patient should not have to spend an unreasonable period traveling to the hospital for treatment. A number of radiation regimens are available from single-fraction treatment to extended courses of treatment lasting 4 weeks. The precise regimen should depend on the life expectancy of the patient. Radiation therapy may also be useful for preventing pathologic fracture in long bones, may obviate the need for surgical repair, and may be used to palliate such emergent or urgent conditions as spinal cord compression and superior vena cava syndrome.

### General Effects of Radiation on Normal Tissues

In addition to its effects on tumors, radiation therapy also produces effects on normal tissues. These effects can be seen during treatment or many years after treatment. Reactions that occur during or soon after treatment are referred to as *acute effects* whereas reactions seen more than 3 months after a course of radiation therapy are referred to as *late effects*. The tolerance of normal tissues to fractionated radiation therapy is a function of the volume of normal tissue or organ being irradiated, the dose that the tissue or organ receives, and the time course of the treatment. For a given dose of radiation, a more protracted course of treatment produces fewer side effects. Because volume is a critical determinant of normal tissue tolerance, new delivery methods, such as IMRT, may be important in reducing normal tissue effects. When chemotherapy and radiation are combined, acute and late effects of treatment can be more severe. Radiation doses are generally lowered in combined modality therapy.

The early and late effects of radiation are generally limited to the area of the body that is being irradiated. One exception to this rule is that some patients undergoing treatment will notice fatigue. This usually begins several weeks into a course of treatment and can persist for several months after treatment. An example of acute and late effects of radiation is seen in head and neck treatment. The acute effect of this treatment may be an inflammation of the mucosal tissues (i.e., mucositis), whereas the late effect may be a dry mouth. Another example of acute and late effects is seen in abdominal radiation. Irradiation of the bowel may produce bowel movement alterations during treatment, whereas a late effect (albeit rare) is a bowel obstruction. Generally, acute effects of radiation require supportive care, and the patient should be reassured that these effects will improve after the completion of treatment. Mucositis is generally treated with hydration, topical anesthetics, and narcotics. Acute skin reactions, which can occasionally be severe, are treated with topical creams prescribed by the radiation oncologist and rarely require antibiotics. Cloth dressings can sometimes worsen these reactions and are generally not recommended.

### Late Effects

It is important that clinicians pay attention to complaints by patients that are referable to an area of the body that has been treated with radiation. This may signify tumor recurrence or the appearance of a late effect of treatment. With more modern tumor imaging and radiation treatment techniques that permit smaller volumes of normal tissue to be irradiated, untoward complications should be reduced in the future. Nonetheless, late effects will be with us for many years to come. In general, tolerance to the acute and subacute effects of irradiation is improved in pediatric patients, but children are susceptible to a wider range and number of late complications.

In contrast to acute reactions, the late effects of radiation are observed from months to years after irradiation. Although the exact mechanism is unknown, late radiation complications have been thought to be related to either vascular endothelial damage or damage to parenchymal stem cells of the irradiated organ. Each

organ appears to have a unique radiation tolerance, and, thus, vascular injury may not entirely explain the long-term effects of radiation. The late complications of radiation therapy are often the most critical dose considerations in clinical practice. [1,36,42,43,50]

Late effects appear to be dependent primarily on the total dose of radiation and the fraction size. [2,32] When the total dose or fraction size is increased, unacceptable late complications can ensue.

In the child, irradiation before full development of various tissues can result in failure of normal development. This is most evident in neurocognitive sequelae in brain tumor survivors. In addition, bone growth may be affected. The severity of growth retardation is related primarily to the age at treatment, the dose of radiation used, and the location treated. Doses of more than 20 Gy generally have a significant effect on bony growth. [42,43] However, the younger the patient, the greater the effect because there is more growth remaining. In brain tumors such as medulloblastoma with the potential for cerebrospinal fluid spread, the entire craniospinal axis is treated. With such treatment, the growth retardation is manifested primarily as a reduction in sitting height. [43] In addition, irradiation to the brain can result in a decrease in growth hormone with resulting short stature.

One of the most devastating late effects of radiation therapy is the development of a treatment-associated second cancer. [88,89 and 90] This event has been well documented in survivors of childhood cancers. By definition, a radiation-associated malignancy must occur after some latency period within the prior radiotherapy volume and be a different histology than the original tumor. A variety of factors influence the risk of a second tumor, including the patient's underlying genetic predisposition, the dose of radiation, the tissue irradiated, the sex of the patient, and the age at treatment. The thyroid gland, for example, appears sensitive to second cancer after low radiation doses. Breast tissue appears to be particularly sensitive in young women who receive radiation to the breast area. [85]

The volume receiving radiation may be critical to the risk of second tumors as well. For example, the current standard in the treatment of childhood Hodgkin's disease is to limit the dose as well as the volume needing radiation by the use of combined modality therapy, with the expectation that the risk of second tumors will be reduced compared to historical wide-field irradiation. Please see Chapter 49 for a more detailed discussion of second malignancies.

## SUMMARY

Radiation therapy has made important contributions to modern cancer therapy. As a single modality, it has produced impressive cure rates at a number of disease sites. In combined modality therapy, radiation can improve cancer control rates that are achievable with surgery and chemotherapy. Modern delivery techniques, such as proton therapy and IMRT therapy, should continue to improve cure rates and reduce the acute and late effects of treatment.

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14

# PRINCIPLES OF IMMUNE AND CELLULAR THERAPY

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## INTRODUCTION

Immunotherapy exploits leukocytes, antibodies, and cytokines for tumor destruction. The potential for target specificity renders this approach highly attractive. Early efforts at immune stimulation using bacterial extracts and BCG have been superseded by more sophisticated approaches built on an expanded understanding of the immune system and tumor cells. Clinical availability of genetically engineered antibodies, cytokines, and tumor vaccines has facilitated the evaluation of the toxicities and therapeutic potentials of these agents. Active immunotherapy attempts to induce effectors with immunologic memory, an assurance of long-term protection that is unique in the armamentarium of medical therapeutics. Nevertheless, during the period of immunosuppression by CA and its treatment, passive immunotherapy can have a distinct role. Despite the increasingly intensive use of traditional antiCA treatment modalities that have been effective in rendering patients clinically free of disease, cure remains elusive for many children. It is likely that the role of immunotherapy is to supplement what dose-intensive treatments have failed to accomplish—namely, the eradication of microscopic disease.

Autoimmune or alloimmune reactions can completely destroy organ systems.[1] Conversely, the catastrophic consequences of a defective immune system are evident in various immunodeficiency states, especially in AIDS. Tumor-specific immunity can be induced *in vivo* (i.e., active immunotherapy) or induced and amplified *in vitro* before administration into a patient (i.e., passive immunotherapy). Tumor cells can stimulate a cellular immune response consisting of helper T cells and CTLs. The helper T cell releases cytokines, recruits other effectors, and regulates antibody production by B lymphocytes (i.e., humoral immunity). CTLs kill targets by cytotoxic granules or programmed cell death. Other effector cells of the immune system, including monocytes, granulocytes, NK cells, and eosinophils, are not by themselves tumor specific, but they can be armed with specific antibodies and be rendered tumor selective, a process called *antibody-dependent cell-mediated cytotoxicity* (ADCC). Tumors can also stimulate the formation of antibodies, which kill by complement activation or by mediating ADCC.

## HUMORAL IMMUNOTHERAPY

### Monoclonal Antibodies

Serotherapy using heterologous serum has been largely replaced by the use of MoAbs ( Fig. 14-1), a technology first introduced by Kohler and Milstein.[2] Through somatic cell hybridization, individual plasma cells, each secreting a monoclonal species of antibody, can be immortalized. MoAb offers multiple advantages (e.g., specificity, precision, purity, and quantity) that can be scaled up in pharmaceutical plants. The clinical utility of MoAb for *in vitro* diagnosis and *ex vivo* purging of bone marrow/stem cells is well recognized. This chapter focuses on their clinical applications *in vivo*. Optimal targeting of MoAbs demands high tumor antigen density with homogeneous expression, antigen persistence on tumor cell surface, noninterference by circulating free antigens, low HAMA titer, minimal cross-reactivity with normal tissues, adequate vascularity to allow tumor penetration, and low RES uptake. In practice, few MoAb-antigen-tumor systems possess all of these attributes.

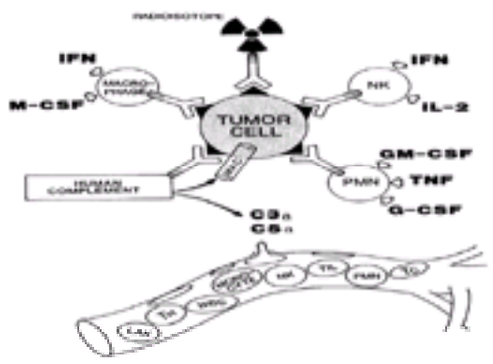


**FIGURE 14-1.** Monoclonal antibody targeted therapy. Antibodies (Y) bind to tumor-associated antigens ( *solid triangles*) and activate complement to form C3a and C5a (chemotactic factors and anaphylatoxins) as well as membrane attack complex (MAC). They also adhere to Fc receptors on leukocytes, including neutrophils (PMN), natural killer cells (NK), and macrophages. G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL-2, interleukin-2; LAK, lymphokine-activated killer cells; M-CSF, macrophage colony-stimulating factor; Tc, cytotoxic T cells; $T_H$, T helper cells; TIL, tumor-infiltrating lymphocytes; TNF, tumor necrosis factor; WBC, white blood cells. (Modified from Cheung NK. Immunotherapy: neuroblastoma as a model. In: Horowitz ME, Pizzo PA, eds. Pediatric solid tumors: pediatric clinics of North America, vol. 2. Philadelphia: WB Saunders, 1991:425–441, with permission.)

#### *Target Antigens*

#### Gangliosides

Gangliosides[3] are acidic glycosphingolipids found on the outer surface of most cell membranes; they are particularly concentrated in gray matter and synaptic junctions in the nervous system, especially during brain development. In serum, gangliosides are found in low concentrations and are transported by lipoproteins. Only trace amounts of gangliosides are found in the cerebrospinal fluid. The lipophilic ceramide portion of the ganglioside is inserted into the lipid bilayer of membranes, and the hydrophilic carbohydrate moieties are exposed to the external environment. They are relatively nonimmunogenic and, although antiganglioside antibodies are found only in neurologic disorders and malignancies, their pathologic significance is unknown. Many tumors have abnormal glycolipid composition and structure. $G_{D2}$, $G_{D3,}$ $G_{M2}$, and O-acetyl-gangliosides have been found in a wide spectrum of human tumors, including neuroblastomas, [4,5] osteosarcomas,[6] soft tissue sarcomas,[7] medulloblastomas, high-grade astrocytomas,[8] melanomas,[9] and small cell lung CAs.[10] They are ideal targets for MoAb because of their high antigen density and persistence on cell membranes. In addition, the only normal tissues with high ganglioside expression are neurons, which are protected from intravenous MoAbs by the intact blood–brain barrier. R24,[11] specific for $G_{D3}$, and 3F8[4] and 14.18,[12] both specific for $G_{D2}$, are examples of MoAbs that have shown successful targeting and antitumor effects in patients with melanoma and neuroblastoma (Table 14-1). KM8969, a recently humanized anti-$G_{M2}$ MoAb, may have clinical potential

given its highly restricted normal tissue distribution. [13]

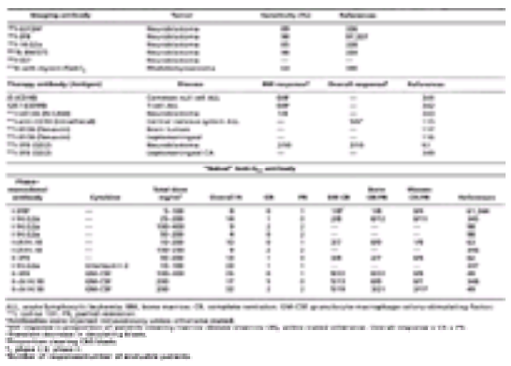

**TABLE 14-1. USE OF MONOCLONAL ANTIBODIES FOR *IN VIVO* DIAGNOSIS AND THERAPY IN PEDIATRIC IMAGING**

### Idiotypes

B-cell tumors express monoclonal surface immunoglobulins that contain idiotypes, [14] which are unique tumor targets. Murine antiidiotypic antibodies, either alone or in combination with IFN-a, IL-2, or chlorambucil, have produced major responses (18% CR) against adult B-cell lymphomas, some of which were durable. [14,15] Tumor regression can be delayed up to 4 weeks after completion of therapy and prolonged with continuing tumor response for 1 year after treatment. [14] HAMA response was uncommon,[14] and the exact antitumor mechanism is still unclear. The persistence of malignant clones despite clinical remission and the emergence of idiotype-negative variants [15,16] have remained obstacles to cure. Although highly successful in adult follicular lymphoma, this strategy is not feasible in pediatric leukemias and lymphomas, in which surface idiotype expression is usually rare.

### Growth Factor and Receptors

Tumor cells proliferate in response to paracrine and autocrine signals. These include insulin-like growth factors and their receptors [17] (in neuroblastoma[18] and rhabdomyosarcoma[19]); nerve growth factor and its receptor TrkA (neuroblastoma)20; p75, or brain-derived nerve growth factor, and its receptor TrkB (neuroblastoma)[20]; or neurotrophin-3 and its receptor TrkC (medulloblastoma and neuroblastoma). [20] MoAbs can potentially stop tumor growth by removing the cytokine or by blocking its specific receptors. However, the ubiquitous nature of these ligands and receptors interferes with their selective tumor targeting. Most clinical experience of receptor-specific MoAb was gathered in adults: anti-IL-2/IL-2R (anti-TAC) in T-cell leukemia and lymphomas; anti-EGF receptor (anti-EGF-R) in lung, breast, bladder, and glial CAs [17]; and anti-HER2/neu/c-*erb* B-2 in breast CA[17] and osteosarcoma.[21] Anti-IL-2R (anti-TAC) MoAbs can target effectively to activated T cells in GVHD and human T-lymphotrophic virus type 1–associated adult T-cell leukemia. [22] Clinical trials using anti-EGF-R antibody in patients with lung CA have shown very few side effects.[17] The antiproliferative effect of anti–EGF-R synergizes with chemotherapy in preclinical models [17] and clinical trials.[23]

### Death-Signaling Receptors

Apoptosis or programmed cell death occurs in T cells during normal thymocyte development and in target tumor cells after CTLs deliver their lethal hit. Fas/APO-1 is a 52-kd cell surface protein capable of inducing apoptosis upon specific antibody engagement in lymphomas and neuroblastomas. In human B-cell lymphoma xenografts, MoAb to APO-1 induces tumor regression histologically suggestive of apoptosis. [24] Because Fas is a ubiquitous death-signaling receptor, however, the clinical utility of Fas-MoAb remains uncertain.

### P-Glycoprotein and Multidrug Resistance

Many human tumors, including neuroblastoma,[25] rhabdomyosarcoma,[26] and osteosarcoma,[26] have overexpression of MDR-1 protein, a P-glycoprotein involved in drug transport and pleiotropic resistance. MRP[27] is also associated with poor clinical outcome in neuroblastoma. MoAbs against MDR-1 or MRP can potentially block the drug efflux pump or directly eliminate cells that overexpress such proteins.

### Tumor Angiogenic Receptors

First proposed by Folkman in 1972,[28] tumor vasculature has become an important target in CA therapy.[29] Both pro-angiogenesis and anti-angiogenesis factors and receptors have been cloned. MoAb to such factors or their receptors [e.g., VEGF receptor, flk-1/KDR (type2)] can inhibit the growth of tumor vessels. [30,31] The antivascular effects of low-dose chemotherapy (e.g., vinblastine) were selectively enhanced when VEGF function was blocked by such MoAbs, [31] producing striking tumor regression in xenograft models.

### Lineage-Specific Lymphoid and Myeloid Antigens

Several lineage-specific antigens on lymphoid cells [CD5, CD19, CD20, CD22, CD25 (IL-2R-alpha), CD52] and myeloid cells (CD33, CD45) have served as useful targets for MoAbs. Most of these antigens except CD20 and CD52 are rapidly internalized when bound to antibody, allowing endocytosis of MoAb conjugates, whether radioisotopes or toxins. CD20, an unglycosylated phosphoprotein, is an optimal target because it does not shed or modulate when bound to MoAb. As a calcium channel, CD20 may regulate B-cell activation. Except for CD20 and CD25, the functions of most of these surface antigens are not entirely clear.

### Intracellular Antigens

Although surface antigens are the usual targets for *in vivo* MoAb diagnosis and therapy, intracellular antigens have also been successfully exploited. TNT-1 is an antibody directed against nuclear histones. Iodine 131–TNT-1 produced regressions of human carcinoma xenografts with no detectable normal tissue toxicity in animals, and it was successful in imaging epithelial carcinoma in humans. [32] Presumably, TNT-1 binds to histones released from necrotic cells, and these antigens are generally not exposed in normal tissues. Nuclear antigens may be attractive candidates for targeted therapy because of their preferential expression in proliferating cells.

### Other Tumor-Associated Antigens

A natural IgM antibody directed at a protein of 260 kd was recently described in normal children. [33] This antibody was absent in infants who had neuroblastoma. When purified, this antibody induced apoptosis of neuroblastoma cell lines *in vitro* and inhibited xenograft growth *in vivo*.[33,34] and [35] Treated xenografts showed massive perivascular complement activation and neutrophil accumulation after 24 hours. Trials are ongoing to test the clinical efficacy of such polyclonal antibodies in treating neuroblastoma. Another antibody, 8H9, directed at a novel glycosylated antigen of approximately 58 kd, was recently described. [36] This antibody reacted strongly with human brain tumors, childhood sarcomas (including osteosarcoma, Ewing's sarcoma, rhabdomyosarcoma, and various sarcomas), and neuroblastomas but was highly restricted in normal human tissues.[36] In biodistribution, it targeted human rhabdomyosarcoma xenografts with favorable tumor to nontumor ratio. [37]

## *Antibody Effector Functions and Conjugates*

### Receptor Cross-Linking and Receptor Blockade

By virtue of their multivalency, MoAbs can cross-link cell-surface receptors activating downstream signals ( Fig. 14-1). In the case of anti-TrkA MoAb, it mimics the

natural ligand, nerve growth factor,[38] leading to trophic signaling. Alternatively, MoAbs can block receptor functions by interfering with binding of the natural ligand (e.g., EGF-R[39] and VEGF-R[30,31,40]).

**Cytophilic Antibodies and Antibody-Dependent Cell-Mediated Cytotoxicity**

Lymphoma, leukemia, neuroblastoma, melanoma, and colon CA cells are effectively killed *in vitro* by lymphocytes, granulocytes, and activated monocytes in the presence of specific MoAb.[41,42,43,44,45 and 46] Human $IgG_1$ or $IgG_3$, and murine $IgG_3$ are most efficient in mediating ADCC. Antibodies function as bivalent ligands, bringing together tumor targets and effector leukocytes. Antibodies can also bind to Fc receptors to activate effector cells. Because the Fc receptors on leukocytes are occupied by normal IgG in blood and tissue fluids, a large amount of MoAb may be required to compete for binding.

To mediate ADCC, FcRIII and adhesion molecule LFA-1 are required for human NK cells. Low concentrations of IL-2 (10 to 100 U per mL) can augment such ADCC. Granulocytes mediate nonoxidative killing of human tumor cell lines *in vitro* (e.g., neuroblastoma in the presence of MoAb 3F8) and require the presence of FcRII, FcRIII, and adhesion molecules CD11b, CD11c, and CD18.[47] Because recombinant human GM-CSF (rhGM-CSF) (2 to 20 ng per mL) and IFN-g can increase granulocyte ADCC *in vitro* by severalfold,[43,44] their local concentrations in tumor tissues may be critical. Because the production of GM-CSF by endothelial cells and monocytes is regulated by activated or immune T lymphocytes, T lymphocytes may play an indirect role in amplifying these antitumor mechanisms. rhGM-CSF induces neutrophilia in patients[48] and may be useful in expanding the effector pool for tumor cytotoxicity. Although MoAb and cytokines appeared to have synergistic antitumor properties *in vitro*, it is uncertain whether their combination will translate into superior clinical results.[49] Antibody sequestration by the cytokine-activated RES can be a limitation. One would hope that cytokines targeted directly to tumor sites via antibody fusion proteins (e.g., ch14.18-IL-2[50] and ch14.18-GM-CSF[51]) would be more selective.[52,53] Activation of T-cell–mediated immunity in rodent tumor studies appeared promising.[54]

Recombinant human M-CSF induces human peripheral blood monocytes to differentiate into large macrophagelike cells *in vitro*. These monocyte-derived macrophages mediate efficient antibody-dependent and antibody-independent cytotoxicities by phagocytosis[46] through the FcRII or FcRIII receptors.[55] In patients, cells of the monocyte-macrophage lineage have been found in some primary tumors and tumor metastases. Local tissue production of factors such as M-CSF may be important in regulating their immunologic functions. In phase I studies, M-CSF induced monocytosis, monocyte cytotoxicity,[56] and thrombocytopenia. However, there was no antitumor effect.

**Complement Activation**

Many tumor cell lines (e.g., leukemia, neuroblastoma, and medulloblastoma) are sensitive to CMC (Fig. 14-1). The terminal pathway of complement activation inserts membrane channels into tumor cells, leading to ionic loss and cell death. However, some tumor cell lines are resistant to complement because of anticomplement surface proteins, including DAF (CD55),[57] homologous restriction factor (CD59),[58] and membrane cofactor protein (CD46).[59] It is also believed that the effect of complement activation extends beyond direct tumor lysis. After complement activation, surface-bound C3b is rapidly cleaved by plasma protease factor I to iC3b. Through CR3 (Mac-1 or $a_Mb_2$-integrin) and CR4 (CD11c/CD18, $a_Xb_2$-integrin) receptors on leukocytes, tumor cells can become opsonized. C3a and C5a, by-products of complement activation, are potent mediators of inflammation[60] and are chemotactic for phagocytic leukocytes. Under normal conditions, leukocytes can be dispatched to any site in the body because of the strong chemotactic signals originating from sites of inflammation. Tumors, however, do not normally release attractants to lure circulating white cells. MoAb (e.g., R24 and 3F8), which activates complement efficiently, has the potential of recruiting effectors to the tumor sites. In addition, C5a enhances lymphocyte function and augments the secretion of IL-1a, IL-1b, and TNF-a from mononuclear cells *in vitro*. These secondary effects can enhance vascular permeability and reduce the transport barriers to MoAb and effector cells.

***Clinical Application of Monoclonal Antibodies***

**First Generation "Naked Antibodies"**

Initial studies of MoAbs have largely focused on their acute toxicities and immunogenicity. In general, toxicities have been tolerable and self-limited. Perhaps the most severe (although self-limited) side effects noted so far have been the pain reaction associated with anti-$G_{D2}$ antibody treatment[61,62 and 63] and the massive cytokine release (leaky capillary leading to hypotension and respiratory distress) associated with anti–T cells MoAb (e.g., patients with chronic lymphocytic leukemia treated with $IgG_{2a}$ MoAb T101). Common acute reactions associated with murine MoAb include fever, blood pressure fluctuations, urticaria, and anaphylactoid reactions and are short-lived. Few long-term toxicities have been observed (e.g., no long-term neurologic toxicities have been seen after intravenous 3F8 treatment in patients followed for as long as 10 years).[64]

Clinical trials using unmodified MoAb in patients with various malignancies have generally been disappointing. Objective responses of large tumor masses are rare. Lymphoid malignancies are more the exception than the rule. Here, antiidiotypic, anti-CD20, and anti-CD52 MoAbs have produced major clinical responses (CR and PR).[14,15,65,66] The chimeric human-mouse anti-CD20 MoAb Rituximab, when used alone or in combination with chemotherapy,[65,67] has produced 55% CR among low-grade lymphomas, with 74% of patients remaining in remission (29 or more months median follow-up). CAMPATH-1H, a humanized rat IgG1 anti-CD53 MoAb, was active against recurrent B lymphoma and T-cell prolymphocytic leukemia.[66] Opportunistic infections, including bacterial sepsis and marrow aplasia, were some of the complications. For neuroectodermal antigens, MoAbs against $G_{D3}$ (R24)[11] and $G_{D2}$ (3F8, 14.18)[61,62 and 63] have been tested most extensively. $G_{D2}$ is particularly relevant for pediatric CA because it is present on a variety of solid tumors. Although the clinical efficacy of anti-$G_{D2}$ antibodies was modest (Table 14-1), response of marrow disease was generally more consistent. During anti-$G_{D2}$ MoAb treatment, most patients had reductions in their serum complement levels, suggesting that complement consumption had occurred. Although the antitumor effect appears to be associated with CMC and possibly ADCC, additional mechanisms are likely in view of the length of time required for response (i.e., 4 to 16 weeks after the initiation of treatment), the continued response long after treatment has ended, and the absence of late relapse after 3 years (Fig. 14-2). Antiidiotype network or T-cell response against antibody-modified tumors could have contributed to the tumor regression.[68,69,70 and 71]

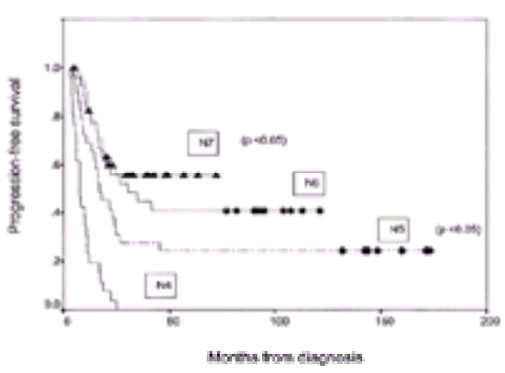


**FIGURE 14-2.** Kaplan-Meier plots of progression-free survival among 121 consecutive patients with stage 4 neuroblastoma diagnosed at age older than 1 year and initially treated at Memorial Sloan-Kettering Cancer Center. A, Adriamycin; C, cyclophosphamide; N4, CAV + ABMT; N5, CAV/PE + ABMT; N6, CAV/PE + 3F8; N7, N6 + 131-I-3F8; P, cisplatin; V, vincristine; E, etoposide.

The clinical role of "naked" MoAb may be in the adjuvant setting for eradicating micrometastases. This was recently demonstrated in patients with colorectal CA[72] and in neuroblastoma.[64] The murine $IgG_{2a}$ 17-1A, specific for gp37–40 on malignant and normal epithelial cells, was administered in a randomized trial to 189 patients with adenocarcinoma of colon or rectum metastatic to regional lymph nodes. At a median follow-up of 5 years, overall death and recurrence rates were substantially reduced. The role of anti-$G_{D2}$ MoAb treatment of minimal residual disease was tested in stage 4 neuroblastoma diagnosed at age older than 1 year.[64] Thirty-four patients were treated with 3F8 at the end of chemotherapy, 23 were in CR, eight in very good partial remission (VGPR), one in PR, and two with histological evidence of marrow disease. Twenty-five of 34 (74%) patients had evidence of disease by more sensitive measures: 14 were $^{131}$I-3F8 scan–positive, nine had marrow disease

by immunocytology, and 12 by reverse transcriptase-polymerase chain reaction of molecular marker *GAGE*. Thirty-eight percent were progression-free (40 to 148 or more months from the initiation of 3F8 treatment). After 3F8 treatment, six of nine patients with positive immunocytology reverted to undetectable, 7 of 12 turned *GAGE*-negative, and all six post–3F8 treatment $^{131}$I-3F8 scans showed resolution or improvement.

### Bispecific Antibodies

Because not all antibodies are cytophilic, by joining two antibodies, one specific for effector cells and one for targets, close contact of effector to target can be achieved.[23] NK cells, granulocytes, or T lymphocytes can be redirected to tumor cells. For example, joining anti-FcRIII (anti-CD16) to antitumor antibodies (anti-CD30) can enhance granulocyte- or NK-ADCC against Hodgkin's lymphoma.[73] The antigen CD3 found on peripheral blood T cells is closely associated with the TCR. MoAb binding to CD3 can activate these T cells to form CTLs. Anti-CD3 conjugated to antitumor MoAbs (e.g., anti-CD19, anti-ErB-2, and anti-CD30) can direct CTLs against human B lymphomas, ErB-2–positive tumors, and HD, respectively.[74,75 and 76] Approximation of leukemia cells (e.g., through anti-CD19, anti-CD20, or anti-CD30) and T-cell costimulatory receptor (anti-CD28) using bispecific antibodies can also enhance specific T-cell immunity. [76,77 and 78]

### Conjugated Antibodies

To enhance the effector functions of MoAbs, conjugation to drugs, toxins, and radionuclides has been explored extensively. Doxorubicin, melphalan, methotrexate, and vinca alkaloids, when conjugated to MoAbs, had limited clinical success. [79] Direct administration of MoAb-melphalan conjugates through the hepatic artery induced mixed response in metastatic colon CA. Tyrosine kinase inhibitors (e.g., Genistein) can be targeted by MoAb (e.g., anti-CD19) to lymphoid malignancies. [80,81] Another novel approach uses MoAb to deliver a covalently conjugated enzyme to the tumor, which can then activate a nontoxic prodrug. [82,83]

***MoAb-Toxin.*** MoAbs have also been used to target toxins derived from plants or bacteria. Once internalized, these toxins are potent inhibitors of protein synthesis. Ricin and diphtheria toxins each consists of two polypeptide chains (A chain and B chain). RTA inactivates the 60S ribosomal subunit whereas the A chain of diphtheria toxin destroys the elongation factor 2 (EF2). RTA or blocked ricin is unable to enter the cells because it lacks a cell attachment site (i.e., the B chain of ricin binds to galactose on cell surfaces). Through a covalent linkage to the MoAb, these toxins can accompany the MoAb-antigen complex when it is endocytosed. Even for poorly endocytosed antibody-antigen systems (e.g., $G_{D2}$), RTA-MoAb has shown striking antitumor effects *in vitro* and in xenografted mice.[84] Pseudomonal exotoxin is a single polypeptide, which causes ADP-ribosylation of EF2, preventing its participation in protein synthesis. The calcium channel blocker verapamil and its analogs have been shown to enhance the *in vitro* cytotoxicity of MoAbs conjugated to *Pseudomonas* exotoxin.[85] In patients with B-cell chronic lymphocytic leukemia, anti-T101-MoAb-RTA had a short half-life and produced minimal toxicity while achieving a transient decrease in circulating tumor cells. It elicited no HAMA and no anti-RTA antibodies, unlike a colon-MoAb-RTA trial in which antibodies were found in all patients. [86] Anti-CD19–blocked ricin induced an overall 21% response (four CR, 20 PR) in 116 patients.[14] The most significant toxicity was vascular leak syndrome, characterized by marked fluid overload, dyspnea, and sensorimotor neuropathies.[87] Deglycosylated RTA devoid of mannose and fucose has been developed to reduce hepatic clearance. With longer serum half-lives, major responses were seen in 25% of patients with lymphoma after anti-CD22-deglycosylated RTA treatment.[85] Recombinant toxins (e.g., pseudomonas exotoxin PE40 and diphtheria toxin $DAB_{486}$), where the cell-binding domains are replaced by single chain Fv or cytokines (IL-2 or IL-6), are in various stages of preclinical and clinical development.[22,85] Pokeweed antiviral protein, a potent inactivator of ribosomes, successfully induced remission in preclinical models when targeted with anti-CD19 [88] and anti-CD7 MoAb.[89] More recently, anti-CD33 antibody conjugated to calicheamicin achieved a high response rate in refractory AML, with tolerable side effects, including fever, chills, and reversible neutropenia. [90,91]

***Radiolabeled Monoclonal Antibody.*** MoAbs have the potential to target tumor-ablative RIT.[92] Radioimaging studies help define the biodistribution of MoAb and estimate the relative amounts of MoAb deposited in various tissues and organs, a prerequisite for therapeutic studies. In preclinical models, ablation of established xenografts is rare but possible [e.g., in neuroblastoma ($^{131}$I-3F8 whole antibody),[93] colon CA [$^{131}$I-$F(ab')_2$ fragments],[94] or lymphoma.[95] Patient responses after RIT have been rarely seen in solid tumors but are seen more commonly in lymphomas and leukemias. Unlike in CMC and ADCC, the bystander effect of RIT is of great importance in understanding the efficacy and toxicity of individual isotopes. Although $^{131}$I has been the most widely used isotope, $^{90}$Y and $^{67}$Cu may have more favorable physical characteristics.

***Imaging Studies.*** Tumor imaging using radiolabeled antibodies can provide valuable information about the extent of disease and for planning targeted radiotherapy. Although quantitation of radioactivity is imprecise with planar imaging, single-photon emission computed tomography, and positron emission tomography in particular, have offered significant improvements.[92] Because radiolabeled MoAbs can inflict significant toxicity, nonspecific uptake in normal organs can degrade therapeutic ratios. In pediatric patients, few antibodies are available for radioimmunoscintigraphy. The ganglioside $G_{D2}$ and gp190 protein, both neuroblastoma antigens, have been the most studied. 3F8 is a murine MoAb specific for $G_{D2}$. In 42 patients with neuroblastoma, $^{131}$I-3F8 localized to neuroblastomas at their primary sites as well as to metastatic disease in the lymph nodes, bone marrow, and bone (Fig. 14-3).[96] A comparison with $^{131}$I-metaiodobenzylguanidine suggests that 2 mCi $^{131}$I-3F8 was more sensitive and specific in detecting metastatic sites of disease. [97] The magnitude of tumor uptake in patients was 0.04% to 0.08% of the injected dose per gram. Imaging studies using 14.2a and ch14.18 antibodies (both anti-$G_{D2}$ MoAb) have also yielded positive results.[98,99] CE7 is a murine antibody against gp190.[100] Its chimeric form, chCE7, is effective in detecting human neuroblastoma.[101,102] More recently, MoAb 8H9 directed at a novel antigen on a broad spectrum of pediatric solid tumors has also shown promise in preclinical models.[37]

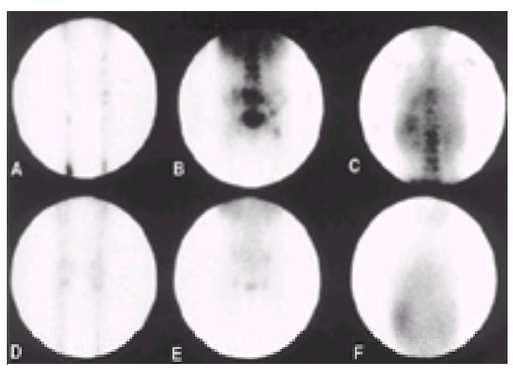


**FIGURE 14-3.** A patient with stage 4 neuroblastoma refractory to myeloablative therapy imaged with $^{131}$I-3F8 before **(A, B,** and **C)** and after **(D, E,** and **F)** 3F8 treatment. **A** and **D**, lower extremities; **B** and **E**, posterior abdomen and pelvis; **C** and **F**, posterior chest. Diffuse disease in spine and pelvis resolved after 3F8 treatment. (From Cheung NK. Immunotherapy: neuroblastoma as a model. In: Horowitz ME, Pizzo PA, eds. Pediatric solid tumors: pediatric clinics of North America, vol. 2. Philadelphia: WB Saunders, 1991:425–441, with permission.)

***Radioimmunotherapy.*** Although many childhood tumors are radiosensitive, the experience of RIT in children is rather limited. In a phase I study, 24 patients (12 boys and 12 girls, 0.3 to 24.2 years of age at diagnosis) with refractory neuroblastoma (23 stage 4, one stage 3 unresectable primary with ascites) at Memorial Sloan-Kettering Cancer Center completed treatment with $^{131}$I-3F8 at seven dose levels, namely 6, 8, 12, 16, 20, 24, and 28 mCi per kg.[92] Twenty-two of 24 patients were rescued with cryopreserved autologous bone marrow; one patient received GM-CSF without marrow rescue; and one died of progressive disease before marrow reinfusion. Acute toxicities of $^{131}$I-3F8 treatment included pain (20 of 24) during the infusion, fever (19 of 24), and mild diarrhea. All patients developed grade 4 myelosuppression. Thyroid uptake despite oral potassium iodide led to hypothyroidism in four patients. Six patients survived longer than 20 months from the time of $^{131}$I-3F8 treatment; none encountered late extramedullary toxicities. Responses were seen in both soft tissue masses and bone marrow.[92] The use of $^{131}$I-3F8 (20 mCi per kg) to consolidate remission was tested in the N7 protocol at Memorial Sloan-Kettering Cancer Center among 35 patients with newly diagnosed (age greater than 1 year) stage 4 neuroblastoma.[103] The median time to engraftment was not substantially different between the newly diagnosed and prior treated group: Approximately 15 days from the day of rescue to reach absolute neutrophil count greater than 500 per µL, 45 days to reach a platelet count greater than 20,000 per µL, and almost 3 months to reach a platelet count greater than 50,000 per µL. Aside from fever and neutropenia plus subsequent infections, there were no extramedullary toxicities other than hypothyroidism, which occurred despite aggressive thyroid protection using oral potassium iodide, liothyronine sodium (Cytomel), and potassium perchlorate. One patient died of infectious complications 2 months after $^{131}$I-3F8 treatment. The overall progression-free survival on N7 is projected to be greater than

50%, compared to 40% in N6 (Fig. 14-2).

Other therapeutic studies using $^{131}$I included anti-EGF-R for grade IV gliomas,[39] anti-CD33 for AML,[104] anti-CR2,[105] anti-CD20 (anti-B1),[106,107] anti-CD37,[108] Lym-1,[109] and anti-CD22 (LL2)[110] in non-Hodgkin's lymphoma primarily in adults. To overcome RES uptake in the liver, a greater than 400-mg dose of naked anti-B1 antibody was used to reduce liver uptake before RIT. Seventy-five cGy was the maximal nonmyeloablative total body dose. Among 28 patients receiving 34 to 161 mCi, 50% achieved CR and 29% PR, with a median response duration of 12 months and median survival of 50 months. HAMA response was found in only 12% of patients. Using myeloablative doses (280 to 785 mCi) calculated to deliver 25 to 27 Gy to critical organs, $^{131}$I-anti-B1 achieved 79% CR and 7% PR,[111] with an overall survival and progression-free survival of 68% and 42%, respectively. Hypothyroidism developed in 60% of patients 6 to 12 months after therapy, and secondary leukemia was not observed. Similarly, myeloablative doses (10 to 31 Gy) of anti-CD37 MoAb also produced 84% CR and 11% PR, with eight patients in continual remission 46 to 95 months after therapy. Extramedullary toxicities were mild at doses of less than 23 Gy, beyond which cardiopulmonary toxicity became dose limiting.[112] In a phase I trial of $^{131}$I-Lym-1 murine antibody, greater than 100 mCi per $m^2$ was myeloablative[113]; 30% had CR and 30% developed HAMA. $^{131}$I–LL2 (anti-CD22)[114] also showed activity against relapsed B-cell lymphoma.

Intraventricular and intrathecal $^{131}$I-anti-CD10 has also been tested in children with central nervous system ALL,[115] whereas intraventricular and intratumor $^{131}$I-81C6 (antitenascin) have prolonged patient survival.[116,117] Clinical trials using intraventricular $^{131}$I-3F8 have also shown favorable dosimetry and minimal side effects in patients with leptomeningeal GD2-positive cancers.[349]

Although $^{131}$I is not the ideal radioisotope for RIT because of *in vivo* dehalogenation, its method of conjugation to proteins is widely accepted and its late toxicity is uncommon. Alternative isotopes [$^{90}$Y-murine anti-CD20 in a phase I/II trial,[118] $^{90}$Y-murine (10 to 54 mCi) anti-idiotypic MoAb,[119] $^{90}$Y-anti-T101 (CD5),[120] $^{90}$Y-anti-TAC (CD25),[121] and $^{67}$Cu-Lym-1[122]] have all produced major responses among patients with advanced lymphoma. Alpha-particles emitting isotopes [e.g., astatine 211 and bismuth 213 ($^{213}$Bi)] carry high linear energy transfer at a short pathlength (0.04 to 0.05 mm) and can destroy tumors more effectively and with more precision. Patient studies of $^{213}$Bi-HuM195 (anti-CD33) have confirmed these predictions.[123] These clinical trials highlighted the utility of RIT in radiosensitive tumors, the limitations of low MoAb tumor penetration, and the dose-limiting myelotoxicity of isotopes. Nevertheless, the relative lack of extramedullary toxicities should encourage further refinement of this targeting technique.

**Pretargeting**

To improve tumor uptake and reduce systemic toxicity, a multistep procedure to pretarget the antibody before the cytotoxic ligand has been successfully used. Generally, a tumor-specific antibody is conjugated to a ligand binder, such as streptavidin or avidin (with high affinity for biotin [124,125]) or ligand-specific antibody (binding to metal chelators such as diethylenetriamine pentaacetic acid,[126] triethylenetetramine,[127] or tetraazacyclododecanetetraacetic acid[127]). In the first step, these conjugates (approximately 200 kd) are allowed to localize to tumors *in vivo*, and any excess is allowed to clear from the blood. The appropriate small radiolabeled ligand (e.g., $^{67}$Cu-triethylenetetramine, $^{90}$Y-tetraazacyclododecanetetraacetic acid, $^{111}$In-diethylenetriamine pentaacetic acid, or radiolabeled biotin) is then injected intravenously in a subsequent step. By virtue of the high affinity interaction, the ligand penetrates tissues rapidly and is taken up strongly by the antibody conjugate at the tumor site. Unbound ligand is quickly excreted through the kidneys. Because of the short transit time of the toxic ligand (radionuclides or toxins), a substantial improvement in the therapeutic ratio without sacrificing the percent dose injected per gram is possible. A three-step approach, which uses biotinylated MoAb followed by avidin/streptavidin then biotinylated chelator, has also been successful.[128] Early clinical trials are showing some promising results.[128,129] This pretargeting concept can also be extended to antibody-directed enzyme prodrug strategies.

**Idiotype Network**

The role of MoAb in pediatric patients can differ substantially from that in adult CAs. Most pediatric CAs are chemoresponsive: MoAb is particularly useful at the time of minimal residual disease. The increasing intensity of chemotherapy can greatly attenuate HAMA response, thereby allowing repeated administrations (e.g., MoAb 3F8 in neuroblastoma).[64] The region of an Ig unique to the antibody is called an *idiotype* (Fig. 14-4). It encompasses the antigen binding site (CDR). Immunization with an antibody (Ab1) specific for an antigen can activate B lymphocytes, producing antiidiotypic antibodies (Ab2) with variable regions bearing the internal image of the first (Ab1) antibody and mimicking the original antigen. Ab2 can in turn induce anti-antiidiotypic antibody (Ab3). This network of antibody responses was first proposed by Jerne.[130] As the immune system recovers from chemotherapy, exposure to tumor-selective MoAb (Ab1) can induce Ab2 and Ab3 and thus bias the recovering repertoire toward the specific antigen network. The potential for biasing the immune system toward specific antigens has been well documented in murine models[131] and human disease states.[132] If true, one would expect such idiotype networks to be particularly useful after intensive immuno- and myelosuppressive therapy. Ab3 was associated with prolonged survival among patients who received $^{131}$I-3F8 imaging pre-ABMT for metastatic neuroblastoma.[133] In a subsequent analysis of patients consolidated with 3F8 in their first or subsequent remission, HAMA response was strongly correlated with prolonged survival and progression-free survival, except in patients with early high titer, which prevented repeat 3F8 treatment.[64] This HAMA may be responsible for the induction of Ab3 and Ab3' (anti-$G_{D2}$) antibody formation, a marker of the host idiotype network thought to be responsible for preventing late relapses after 3 years (e.g., N5, N6, and N7 in Fig. 14-2).[71] Similar observations suggestive of idiotype network were previously reported after MoAb treatment with 17-1A (colon CA)[69,134] and CA125 (ovarian CA).[135]

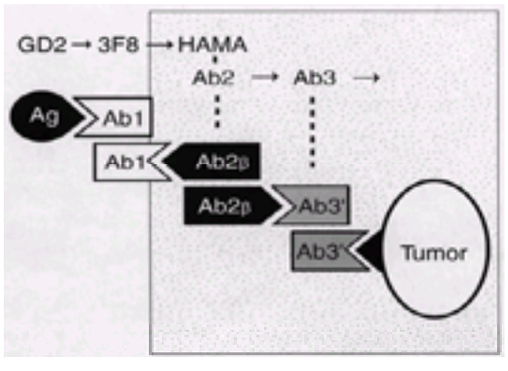


**FIGURE 14-4.** Idiotype network. Antiidiotypic antibodies (Ab2) are a subset of the human antimouse antibody (HAMA) response. Among the Ab2, a subset (Ab2b) bears the internal image of Ab1 (3F8), mimicking the original antigen (Ag). Ab2 can induce anti-antiidiotypic antibodies (Ab3), a subset of which (Ab3') reacts with the original target tumor antigen $G_{D2}$. Shaded box represents the patient.

***Genetically Engineered Antibodies***

Genetically engineered antibodies with potential use in clinical oncology are shown in Figure 14-5.

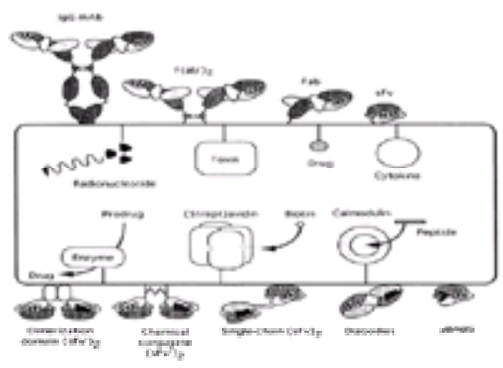

**FIGURE 14-5.** Antibody derivatives with potential use in clinical oncology; heavy chains ( *solid black*), light chains (*light shading*), and complementarity-determining regions (CDRs) (*circles, open or solid*). Progressively smaller antibody molecules can be produced by chemical or recombinant methods ( *top*). Effector molecules (*inside box*) can be conjugated chemically or fused genetically to these recombinant antibodies to provide novel effector functions. Bivalent and bispecific antibodies (*bottom*) can also be made. cBABS, chimeric bispecific antibody–binding sites, in which the bottom loops of sFv, are replaced genetically by the CDRs from antibody of a second specificity[334]; diabodies, bispecific sFv[335]; mAb, monoclonal antibody; sFv and scFv, single chain Fv. [From George AJT, Spooner RA, Epenetos AA. Applications of monoclonal antibodies in clinical oncology. *Immunol Today* 1994;15(Dec):559–561, with permission.]

### Humanization and Chimerization

Human MoAb, being less antigenic in patients, should be superior to mouse MoAb if multiple injections are planned. Success in making human hybridoma has so far been limited by poor fusion efficiency with myeloma partners. The specificity and affinity of an antibody is determined by the variable region, which carries within it small hypervariable sequences called *complementarity-determining regions* (CDRs). These variable regions from murine MoAbs can be genetically joined to human Ig constant domains, producing chimeric MoAbs (e.g., ch14.18 specific for $G_{D2}$).[136,137] A more refined approach uses CDR sequences to graft into human Ig genes.[138] Besides rendering such antibodies humanlike and thus less immunogenic in patients, different Fc can now be selected or modified for a particular immune function. CAMPATH-1 is a rat MoAb that reacts with an antigen expressed by human lymphocytes and monocytes. The CDRs of CAMPATH-1 have been grafted into human heavy and light chain genes to create a series of humanized MoAb of human IgG1, 2, 3, and 4 subclasses. Humanized CAMPATH-1 has been tested *in vitro* for marrow T-cell depletion to prevent GVHD in allogeneic bone marrow transplant and in patients with non-Hodgkin's lymphoma with no detectable neutralizing antibody response.[138] Similar successes in circumventing the HAMA response was reported in other clinical trials using humanized antibodies (e.g., anti-CD33 in AML) [139] and anti-HER2/neu in metastatic breast CA.[140] Most recently, transgenic mice engineered with human Ig loci containing the majority of the human antibody gene repertoire in nearly germline configuration have succeeded in making fully human monoclonal antibodies of both the IgMk and IgGk classes. [141]

### Functional Modifications

With further refinement, the affinity and specificity of an antibody can also be modified by manipulating nucleotides within or around the CDRs. [142] Joining antibody to cytokines (e.g., IL-2,[143] GM-CSF,[144] and IL-12[145]) can further enhance effector functions.[146] In a murine model, antibody targeted IL-2 to eradicate hepatic and pulmonary melanoma metastases in severe combined immunodeficiency mice.[147] Even the Fc regions of MoAb can be exploited by grafting onto virus-binding proteins (e.g., the gp120 binding site of CD4) to produce an immunoadhesin that binds to HIV-infected cells for ADCC. [148]

### Single Chain Fv

Antibody fragments, because of their smaller size, penetrate tumors better than the whole Ig molecule. The single chain Fv (scFv) fragment is a single chain containing the $V_H$ and $V_L$ chains joined by a linker peptide, synthesized by *Escherichia coli* or eukaryotic expression systems such as myeloma cells.[149] ScFv can be synthesized from the complementary DNA of existing myelomas or directly from phage display technology. [150] The binding characteristics of the scFv fragment, including affinity and specificity, can be made similar to those of the whole Ig. [151] Its clearance is more rapid (5 to 15 times faster than the Fab or whole Ig), whereas its tumor uptake is comparable to that of Fab. ScFv fragment of anti-TAC MoAb has been fused genetically to a modified portion of the pseudomonal toxin PE40, which lacks a cell binding site and is nontoxic when free. [152] This novel conjugate killed IL-2R–positive cells but not IL-2R–negative cells *in vitro*. Genetic fusions of scFv to itself (oligomers for improved avidity), to other scFv (bispecific antibodies), to streptavidin (for pretargeting followed by radiolabeled biotin), to enzymes that activate prodrugs, and to other proteins have greatly expanded the repertoire of the antitumor armamentarium ( Fig. 14-5).[151,153] The possibility of producing these recombinant antibody-based proteins in plants will facilitate clinical development of these novel agents. [154]

### T Bodies Can Redirect Lymphocyte against Human Tumors

With the advent of efficient retroviral gene transduction methods, antibody-derived fusion genes can now be introduced into potent killer cells to redirect them specifically to tumor cells.[155] Previous successes of adoptive T-cell therapy (see the section on cellular immunotherapy) exploited the strength of human immune response against viral antigens, where T-cell clonal frequency is high. They are ideal targeting vehicles because they home appropriately to tumors and carry out their cytotoxic functions. They can amplify the antitumor response by recruiting other immune cells. When provided with the primary activation signal (e.g., through engagement of the TCR) and a costimulatory signal (e.g., through CD28), both helper T-cell expansion and cytolytic T-cell expansion are sustained. When transduced into T cells, ScFv are called *T bodies*. Chimeric immune receptors joining tumor-selective ScFv to T-cell signal transduction domains (e.g., Fc-gamma-chain or TCR-zeta-chain[156,157] and CD28[158]) can couple antigen-specific tumor recognition with T-cell activation. When ScFv recognizes tumor cell–surface epitopes, the chimeric immune receptor triggers the release of cytokines, tumor lysis, and tumor rejection. [155,157,159,160,161] and [162] This approach has many advantages. It bypasses the requirement of tumor MHC for T-cell activation. For tumors like neuroblastoma, their downregulation or absence of MHC can disable classic T-cell–based strategies. In addition, human tumor targets often lack costimulatory molecules (e.g., CD80) or overstimulate inhibitory receptors (e.g., CTLA4 on T cells). Furthermore, chimeric immune receptor approach can greatly expand the list of potential antigen targets, many of which are exquisitely specific and most of which have never been targeted by CTLs. Although this strategy uses peripheral blood T cells, it does not depend on preexisting antitumor immunity in CA patients. T bodies have been tested in several tumor models. In ovarian CA, a chimeric construct of scFv against neu/HER[156,157] and human IgGFcR-gamma chain was cloned into retroviral vector for gene transfer into T lymphocytes. GM-CSF secretion and CTL activity against the tumors were detected. [155,156] Anti-ErB2R-Fc-gamma was successfully introduced to retarget CTLs.[159,163] ScFv-Fc(epsilon)RI-gamma chain was used to arm CTL against renal cell carcinoma. [161] Although scFv gene transduction puts a new recognition unit on a lymphocyte to guide its trafficking to the tumor, lymphocytes can be preselected for specific functions such as T helper, CTL, Th1, or Th2. It can also carry genetically engineered properties, such as the ability to secrete cytotoxic cytokines, as was done with TIL cells [164]; to secrete tumor-selective toxins[165]; to metabolize or activate prodrugs (e.g., thymidine kinase and gancyclovir [166] or P450-2B1 that converts cyclophosphamide to 4-hydroperoxycyclophosphamide[167]), or to resist alkylators (e.g., carmustine[168]) or even MDR-1–regulated drugs.[169] The limitation of low clonal frequency of adoptive T cells in TIL and LAK strategies may now be overcome by the T-body approach.

### Diabody and Bispecific Antibodies

Multivalent recombinant antibody fragments have achieved high-avidity binding and novel combinations of specificities. [170] In scFv, $V_H$ and $V_L$ are joined by a linker peptide. A linker of greater than or equal to 12 residues produces predominantly monomers. A linker of three to 12 residues disables scFv folding, forcing its association to form bivalent dimer (diabody of 60 kd).[171] At linker length of less than three, trimers (triabodies, 90 kd) or tetramers (120 kd) predominate, with increasing binding avidity.[171] Most important, compared to monomers (25 kd), molecules of 60 to 120 kd have optimal retention and clearance properties *in vivo*. When the four variable domains ($V_H$ and $V_L$) derived from MoAb of different specificities are linked together (tandem diabody), a bispecific recombinant antibody of 114 kd is created (cross-linking $CD19^+$ malignant B cells and $CD3^+$ T cells).[172] Diabody strategy can also combine antibody fragments specific for different epitopes on the same antigen (e.g., VEGF-R2).[173]

### Humoral Cancer Vaccines

#### *Tumor Antigen Vaccines*

Most antigens can induce antibodies only with the help of T cells. Other antigens (including many carbohydrates) induce immunity without T-cell help, but the antibody response is generally restricted to the IgM class, and without persistent antigen, it is short-lived with no secondary responses. Antibody response to tolerated antigens resembles that of T-independent antigens. If T-cell help is reestablished in tolerant hosts by adoptive immunity, however, the response to these same antigens can be strengthened. Many tumor antigens are believed to lack helper T-cell epitopes (e.g., carbohydrates or glycolipids) and thus are often tolerogens or poor immunogens. Tumor antigens from chemically or virally induced tumors are exceptions. For example, methylcholanthrene- or polyomavirus-induced tumors can sensitize mice against subsequent tumor engraftment. Tumor rejection is mediated and adoptively transferable by T lymphocytes. It is augmented by bacterial products injected into growing tumor nodules or mixed with irradiated immunizing tumor cells.[174] Immunogenicity of cells can also be increased by mutagens,[175] viral or chemical antigens

(i.e., xenogenization),[176] enzymatic unmasking with neuraminidase,[177] or transduction of cytokine/costimulator genes (see the section Cellular Immunotherapy).

### *Tumor/Tumor Antigen Selection*

To identify the tumor antigens for humoral immunity, a serologic approach called *autologous typing* has been employed using autologous tumor cell lines (to avoid alloantibodies) to screen for antitumor serum antibodies in adult patients with melanoma, renal CA, astrocytomas, and leukemia. At least three types of antigens have been defined: (a) mutated antigens restricted to tumor cells, such as EGF-R in glioma[178]; (b) antigens on tumors of common origin but highly restricted among normal tissues, including differentiation antigens such as gangliosides $G_{M2}$, $G_{D2}$, and $G_{D3}$[179] found in neuroectodermal tumors, a-fetoprotein in hepatomas, as well as CEA, TF, and sialylated Tn[180] expressed on a variety of epithelial carcinomas; and (c) antigens widely distributed among normal and malignant cells. The relative contribution of humoral versus cellular immunity in tumor surveillance in patients remains unclear.[181] Furthermore, specific antibodies can inhibit antitumor response by blocking CTL target antigens.[182] Because circulating IgM antibodies specific for selective tumor-associated antigens (e.g., $G_{M2}$, TF, and sialylated Tn) have correlated strongly with longer survival in patients with melanoma and epithelial CAs, a substantial effort has been devoted to the development of humoral vaccines.[183]

### *Carrier Proteins and Adjuvants*

To enhance immunogenicity, covalent attachment of antigen to carrier proteins [e.g., keyhole-limpet hemocyanin (KLH)] to induce T-cell immunity may be required, similar to the experimental models of autoimmune diseases.[183] A less specific approach in increasing immunogenicity uses adjuvants to activate APCs (e.g., macrophages) and T lymphocytes. Examples of such adjuvants include BCG, muramyltripeptide, muramyldipeptide, monophosphoryl lipid A (an analog of endotoxin), Detox (a combination of monophosphoryl lipid A and mycobacterial cell wall skeletons),[184] pluronic triblock copolymers (e.g., L121), and saponin QS-21.[185]

Adjuvants have been incorporated into tumor vaccines to heighten the antitumor humoral response. Early clinical trials combined whole tumor cells or fractions thereof with BCG, complete Freund's adjuvant, Detox, or xenogens. Many of these studies included concomitant chemotherapy without prospective randomization. Therapeutic effects were uncommon (fewer than 10% of the trials showing response) or difficult to reproduce.[181,183] The biochemical/molecular identities of the tumor antigens in these vaccines were uncertain. Immune responses consisted mainly of tumor-nonspecific antibodies against HLA, viral antigens, fetal calf serum, or other common tissue antigens. Specific antibodies have been reported in a high percentage of melanoma patients receiving purified gangliosides in vaccine trials, especially when KLH carrier and QS-21 adjuvant were used.[183]

### *Antiidiotypic Antibodies as Vaccines*

An alternative to using tumor antigens as vaccines exploits antiidiotypic (Ab2) antibodies.[186] Immunization with Ab2 antibodies can induce anti-antiidiotypic antibodies (Ab3) that can cross-react with the original target tumor antigen (Fig. 14-4). As antitumor vaccines, Ab2 antibodies have advantages over native antigens (e.g., carbohydrates) because they induce better T-cell help and stronger antibody response. Because they can be easily manufactured and modified by genetic engineering, they are preferable to difficult chemical synthesis (e.g., complex carbohydrates). Antiidiotype vaccines have been used successfully in experimental infections and tumors and are being evaluated in patients with colon CA (against CEA),[187] melanoma (against $G_{D2}$),[188] and lymphoma. An alternative strategy unique for B-cell lymphomas exploits the clonal expression of cell surface idiotypes. Immunizing patients with their tumor-specific autologous idiotype proteins conjugated to KLH has produced humoral and cell-mediated antiidiotypic responses as well as tumor regressions.[189]

## Cytokines for Cancer Immunotherapy

Cytokines have pleiotropic effects on the immune system. They stimulate the proliferation of effector cells, augment effector cells' tumoricidal properties, enhance the APC function of monocytes and macrophages, and modulate target antigen expression on tumor cells. The use of cytokines for CA immunotherapy is primarily focused on T-cell and NK-cell activation by ILs and IFNs, APC activation (also see the section Cellular Immunotherapy), tumor growth inhibition plus tumor lysis, and granulocyte/macrophage activation of ADCC (also see antibody functions in the section Monoclonal Antibodies).

### *Interleukin-2*

IL-2 is released by T cells following antigen encounter.[190] It binds to a bimolecular receptor complex (i.e., 55-kd alpha chain and 70-kd beta chain) expressed by T cells stimulated by antigen or mitogen. NK cells and large granular lymphocytes bear only the beta chain receptor, which binds IL-2 slowly and with less affinity. After activation by IL-2, however, NK cells express TAC (alpha chain) on their cell surface. IL-2 stimulates lymphoid proliferation *in vitro* and *in vivo*. In preclinical models, IL-2 can expand antigen-specific T lymphocytes *in vitro* before adoptive transfer into tumor-bearing animals. It can also expand lymphocyte pools in tumor-bearing animals. Tumor specific T cells can be found in spleen, in lymph nodes, or as TIL. Because most spontaneous syngeneic tumors are poorly immunogenic, however, tumor-specific T cells in patients are usually rare and difficult to boost.

Although NK cells can kill tumor cell lines *in vitro*, most fresh tumors are resistant to unactivated NK cells. After exposure to high concentrations of IL-2, however, a heterogeneous population of peripheral blood–derived NK (and probably other lymphoid) cells acquire the LAK cells' ability to lyse fresh tumor cells, endothelial cells, lymphocytes, monocytes, and cells that are virally infected or hapten modified. Unlike NK cells, LAK cells are IL-2 dependent.[190] Distinct from that of T cells, their cytotoxicity is not MHC restricted, and they lack mature T-cell markers. IL-2–activated NK and LAK cells have increased surface Fc receptors that enhance their ability to mediate ADCC. The best tumor responses in animal models were achieved if IL-2 treatment was administered over several days or weeks, and when higher doses of IL-2 were used.[190]

IL-2 administered intravenously has produced significant biologic effects.[191] It causes rapid egress of lymphocytes into tissues, resulting in lymphopenia during IL-2 treatment and rebound lymphocytosis after IL-2 is stopped. It increases the synthesis and release of IL-2 receptors, induces secondary cytokine (e.g., IFNs, TNF-a, IL-6) release into blood, decreases PMN chemotaxis and Fc expression, increases endothelial-leukocyte adhesion molecules on endothelial cells, produces eosinophilia, and activates LAK activity. Side effects include nausea, vomiting, diarrhea, rash, thrombocytopenia, cholestasis, increased sepsis, and capillary leak syndrome, leading to pleural and pericardial effusion, neurologic deficits (disorientation, somnolence, coma), peripheral edema, oliguria, and weight gain. Severe cardiopulmonary toxicities in children have been observed at doses greater than or equal to $3 \times 10^6$ units per $m^2$ per day. Continuous IL-2 infusion is less toxic than bolus therapy. Most studies report a 20% remission rate (usually a partial response lasting a few months) in patients with renal cell carcinoma or metastatic melanoma. In AML, IL-2 administration after myeloablative chemotherapy may prolong remission[192]; however, it is not effective against ALL. Except for minor responses (e.g., transient decrease in urine catecholamines and disappearance of skin nodules) in patients with neuroblastoma treated with IL-2 alone after marrow transplantation,[193] no antitumor effect of IL-2 was found in four pediatric phase I trials, irrespective of drug schedule (continuous infusion versus two to three weekly bolus injections).[194,195 and 196]

### *Interferons*

IFN-a and -b[197] can activate NK and monocyte functions and are active clinically against a variety of adult CAs, including bladder CA, AIDS-related Kaposi's sarcoma, renal cell carcinoma, malignant melanoma, solid tumors, hairy cell leukemia, chronic phase CML, low-grade NHL, multiple myeloma, and cutaneous T-cell lymphoma.[198] However, complete responses are rare. The impact on survival has been small, and no cures have been achieved. The combination of 13- *cis*-retinoic acid and IFN-a has produced major responses in 47% of patients with advanced squamous cell carcinoma of the cervix.[199] However, attempts to show a direct correlation between laboratory assessments of NK, ADCC, and T-lymphocyte activities with clinical efficacy have been unsuccessful.

IFN trials in children have been limited. *In vitro*, IFN-g up-regulates MHC class I and adhesion molecules in neuroblastoma.[200,201 and 202] In neuroblastoma patients[203] treated with 0.05 to 0.10 mg per $m^2$ IFN-g intravenously three times each week for 4 weeks, a transient increase in NK activity and expression of MHC antigens in neuroblastoma cells was seen in four of six patients. However, no significant clinical response was observed. Children with recurrent brain tumors treated with IFN-b (50 to $600 \times 10^6$ IU per $m^2$ three times per week for 14 weeks) demonstrated a partial response radiographically in 4 of 21 patients (including two with brain stem glioma and two with anaplastic astrocytoma), with a median duration of response of 4 months.[204] The efficacy of IFN-a in osteogenic sarcoma, ALL (two partial responses among 31 patients), and posttransplant lymphoproliferative disorders needs confirmation.[205,206 and 207]

Common toxicities of IFN treatment are flulike symptoms, including fever, chills, rigors, myalgia, and malaise, which usually respond to acetaminophen. Labile blood pressure at the initiation of treatment can be lessened by a slow dosage escalation. Neutropenia is occasionally dose limiting. Other side effects include hepatic enzyme elevations (common at the outset, usually reversible even with continued treatment), thrombocytopenia, headache, minor cognitive impairment with a decreased ability to concentrate, and distal paresthesias. The dose-limiting toxicities of IFN-b in children with brain tumors include marrow suppression, seizures, stupor, and coma.

### *Tumor Necrosis Factor*

TNF-a[208,209] inhibits the proliferation of 30% to 40% of malignant cell lines. It exerts pleiotropic effects on phagocytes, endothelial cells, and the immune system: it activates granulocytes and monocytes, up-regulates adhesion molecules on endothelium, stimulates B- and T-cell proliferation, and up-regulates IL-2 receptor expression on T cells. Toxic reactions include fever, rigor, nausea, vomiting, slight depression of platelet and granulocyte counts, neurologic deficits (e.g., confusion and seizures), elevation of serum hepatic enzymes, blood urea nitrogen, and creatinine, as well as dose-limiting hypotension. In animals, intravenous TNF-a causes tumor necrosis and cachexia, but its *in vivo* activity in humans has been limited even at maximum tolerated doses. In contrast, the use of TNF in isolation perfusion technique for extremity melanoma or sarcoma, especially in combination with IFN and melphalan, has substantial clinical activity (90% CR). [210] Clinical trials to explore the role of TNF in regional perfusion therapy are being pursued, and TNF gene–modified TIL cells have also been tested in melanoma patients. [211]

### Ex Vivo *Use of Cytokine and Cytokine Fusion Proteins*

The clinical benefit of cytokines in immunomodulation and in directly inhibiting or killing tumor cells *in vivo* has been modest. However, the use of cytokines for *ex vivo* maturation and expansion of APC (GM-CSF, IL-4, TNF-a) or T cells (IL-2) before adoptive cell therapy is a clear example of how cytokines may be most optimally used. Alternatively, cytokines (e.g., IL-2 or IL-12) targeted to tumor sites using chimeric antibodies or scFv provide yet another approach to activate effector cells in the tumor microenvironment. Among the new generation of ILs, IL-12 has clinical potential because of its ability to augment cytolytic activity and to stimulate T helper cells.[212] IL-12 also induces T-dependent protective immunity in syngeneic models of murine neuroblastoma. [213] Chimeric MoAb-IL-12 fusion proteins have shown promising antitumor effects in mouse models.[145,214] Nevertheless, the broad distribution of cytokine receptors on normal cells and tissues can shorten the half-lives of most administered cytokines and limit their clinical efficacy. [215] In addition, systemic toxicity remains a limiting factor when high doses are used, especially for cytokines that increase vascular permeability, sometimes causing fluid leak and major organ insufficiencies.

## CELLULAR IMMUNOTHERAPY

The cellular immune response, which is designed to kill virus-infected cells before they are able to release the infectious virus, can also prevent the malignant outgrowth of tumor cells. There is now abundant evidence that tumors, even those not associated with viruses, can be immunogenic—that is, they frequently express antigens in a form recognizable by the host immune system. Nonspecific killer cells, such as NK cells and LAK cells, recognize abnormalities at the cell surface, such as low expression of MHC class I molecules or carbohydrate anomalies. CTLs recognize “foreign” peptides derived from cytosolic proteins and presented at the cell surface by MHC molecules. The immune system may perceive proteins as foreign not only if they are expressed by a virus but also if they contain a novel peptide created within an oncogenic fusion protein, or if they are expressed at higher than normal levels or represent proteins not normally expressed in adult tissues. This ability has been demonstrated not only in experimental animals but also for spontaneously occurring human tumors. Despite their expression of immunogenic antigens, some tumors evade immune responses by mutating those antigens, by releasing inhibitory cytokines, or by interfering with the antigen-presentation pathway; others simply down-regulate cell adhesion or costimulatory molecules, resulting in failure to activate specific immune responses. Immune defenses are much more likely to fail if immune function is compromised (e.g., after organ or stem cell transplantation or in patients with immunodeficiency disorders). Improved understanding of immune escape mechanisms has led to strategies that seek to counteract the immune evasion tactics of tumor cells. This section discusses the mechanisms and results of current modes of cellular immunotherapy, which aim to generate responses to inadequately presented antigens or to boost existing responses with the goal of recruiting a larger “army” of immune effectors.

### Requirements for the Activation and Expansion of Antigen-Specific T Cells

The activation of antigen-specific T lymphocytes is a multistep process requiring antigen-specific triggering of the TCR complex on the T cell and additional signaling via costimulatory molecules (Fig. 14-6). The TCR is triggered by the specific recognition of foreign peptides complexed with MHC class I or class II molecules at the cell surface. CTLs classically recognize peptides presented on class I molecules. Class I peptide loading occurs in the endoplasmic reticulum and requires proteasome-mediated antigen processing in the cytosol (Fig. 14-7). Thus, for class I presentation, antigens must gain access to the cytosol. This is usually a prerogative of endogenously expressed proteins, because even professional APCs have a limited capacity to phagocytose soluble antigens and process them for MHC class I presentation.[216] Proteins can gain access to the cytosol by other means, however (e.g., by virus transduction or physical transfection methods). [217] In addition to peptide/MHC binding, a key costimulatory signal results frombinding of the CD28 receptor on T cells with CD80 (B7-1/BB1) and CD86 (B7-2/B70) ligands on the APCs (Fig. 14-6). Inhibition of this pathway in the presence of antigenic stimulation results in T-cell anergy. [218] The CD40–CD154 (CD40L) pathway was also shown to contribute to the regulation of T-cell activation, both by independently costimulating T cells and at least in part by up-regulating CD80/CD86 molecules on APCs.[219] Adhesion molecules, such as LFA1, LFA3, and ICAM-1, are also important in the initial binding of CTLs to APC. For example, LFA1 on target cells lowers the threshold amount of antigen necessary for T-cell activation. [220] Whether adhesion molecules have costimulatory effects remains unclear. [221,222] Tumor antigens may be presented to immune effector cells by the tumor or virus-infected cell itself, but cross-priming by professional APCs is thought to be more common, because most tissues do not have an adequate APC phenotype. Thus, dying tumor cells are taken up by local professional APCs, which then present the tumor antigens to the immune system. The mechanism of cell death is thought to play a critical role in this process: Whereas DCs phagocytose both apoptotic and necrotic cells, the latter induce DC maturation, an important step in T-cell activation. [223,224,225] and [226] Finally, CTLs must receive the appropriate help before expansion can occur. Antigen-specific T helper cells with Th1 activity must be coactivated by the APCs. [225] Th1 cells release Th1 cytokines, such as IFN-g and IL-12, which are also necessary for CTL activation, and IL-2, which is necessary for CTL expansion. [227] A tumor cell that induces the secretion of Th2 cytokines, such as IL-4 and IL-10, may promote antibody instead of CTL responses.

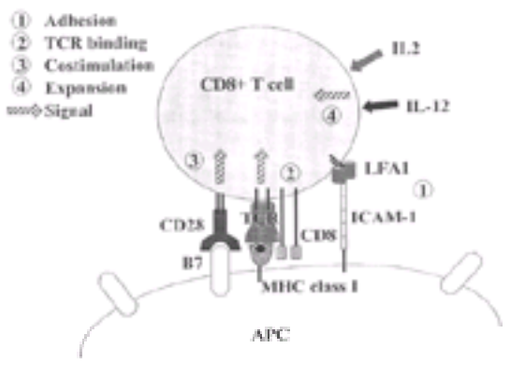


**FIGURE 14-6.** Requirements for T-cell activation and expansion. Adhesion molecules, such as leukocyte function antigen 1 (LFA1) on the cytotoxic T cell (CTL) and intracellular adhesion molecule 1 (ICAM-1) on the target cell facilitate the interaction of CTL and tumor cell (step 1). In the second phase, the T-cell receptor (TCR) must bind to its cognate peptide presented by the appropriate HLA molecule (step 2). In the third phase, the T cell must receive costimulation, commonly by binding of B7 (CD80/86) on the target cell to CD28 (step 3). Other receptor ligand pairs, such as CD137 or CD40L and their ligands, can fulfill this function. If this signal is not received, the T cell can be energized. In step 4, the T cell must be exposed to growth factors such as IL-12 (released by DCs) and IL-2 (released by T helper 1 cells). APC, antigen-presenting cell; MHC, major histocompatibility complex.

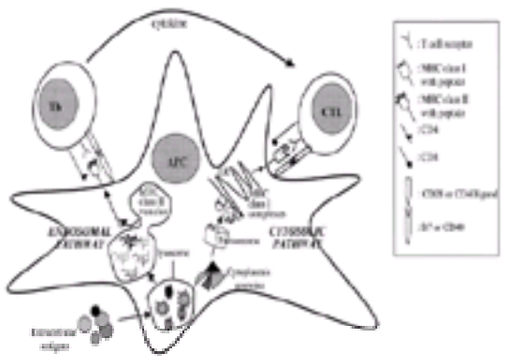

**FIGURE 14-7.** Class I and class II antigen-presenting pathways. $CD8^+$ T cells recognize peptides expressed on major histocompatibility complex (MHC) class I molecules. For MHC class I loading, intracellular proteins are degraded in the cytosol through the ubiquitin pathway. The resultant peptides are carried by transporter-associated proteins 1 and 2 (TAP1 and 2) to the endoplasmic reticulum (ER), where they are loaded onto nascent class l molecules and carried to the cell surface. By contrast, class II peptides are usually derived from extracellular proteins that are phagocytosed by antigen-presenting cells (APCs) and digested in endosomes, where they are loaded onto class II molecules. At the cell surface, they are recognized by $CD4^+$ T cells (CTLs). Th, T helper.

### Immune Evasion Tactics and Their Circumvention

As mentioned earlier, tumor cells use a variety of tactics to avoid immune responses. They can down-regulate critical cell surface activation molecules or release inhibitory cytokines and chemokines that inactivate not only T helper cells and CTLs but also local professional APCs that otherwise might be able to compensate for the tumor cell's poor ability to present its own antigens. A tumor cell that presents a tumor peptide–MHC complex but does not provide a costimulatory signal will anergize peptide-specific T cells.[228] Conversely, a tumor expressing tumor antigens and costimulatory molecules may not present the tumor peptides because of interference with the antigen-processing pathway. The peptide transporter molecules TAP1 and TAP2 are inhibited in Burkitt's lymphoma and melanoma, whereas anchoring of class I molecules in the endoplasmic reticulum is a mechanism commonly used by viruses.[229,230] Tumors may also inhibit cross-priming by professional APCs by secreting cytokines such as IL-10, which down-regulates MHC class II molecule expression on macrophages and DCs and prevents their release of inflammatory cytokines.[231] Even if a CTL receives appropriate activating signals from the tumor cell or APC, the tumor may inhibit or divert the expansion phase. Many tumor cells secrete TGF-b, which inhibits CTL activation in part by inhibiting early signaling events essential to the induction of IFN-g, IL-12, and TNF-a.[232,233] Others secrete chemokines that selectively recruit Th2 cells, which in turn inhibit the Th1 CTL response, perhaps explaining why some CA patients, such as those with neuroblastoma or HD, produce tumor-specific antibodies.[234,235,236] and [237] It seems likely that most tumors in immunocompetent individuals rely on a combination of immune evasion strategies.

Selecting an effective immunotherapy for a particular tumor requires a full understanding of the immune evasion strategies the tumor uses. If a tumor cell does not present a target antigen on its surface because of defective TAP expression, it will not respond to tumor-specific CTLs, regardless of the number of cells infused. If it secretes inhibitory factors, T cells activated by vaccination may fail to expand or will be diverted along the Th2 pathway despite an initial tumor-specific response. For example, although RS cells in HD express viral target antigens and have a good antigen-presenting phenotype, they secrete inhibitory cytokines. This disease may therefore require the use of adoptive therapy with *ex vivo*–expanded CTLs to circumvent this *in vivo* inhibition. Similarly, vaccine strategies often fail in immunodeficient or immunosuppressed individuals who cannot mount an adequate immune response to the vaccine. The best clinical results of immunotherapy with CTLs were obtained in immunodeficient patients for diseases effectively controlled in normal individuals.[238] and [239] Indeed, the EBV-associated lymphomas that occur with increased frequency after solid organ or stem cell transplantation can be prevented or treated simply by infusing of EBV-specific CTLs.[240] and [241]

### Induction of Cytotoxic T Cell Responses by Vaccination

Vaccination has been a powerful tool to combat viral infections. Most protein vaccines elicit only humoral (antibody) immune responses, which can prevent viral infections or render them subclinical by neutralization. For other viruses, neutralizing antibodies are insufficient to protect the host against infection or to prevent the reactivation of latent viruses. For example, serum antibodies that effectively neutralize EBV do not prevent reinfection with new viral strains via mucosal routes, nor do they prevent the outgrowth of EBV-associated lymphomas in patients. These failures can be attributed to the proteins expressed by EBV-associated tumors, which either have a nuclear location or occupy an integral position within the surface membrane and therefore are not accessible to antibodies. In fact, only a few tumor-specific antigens have been identified on the surface of tumor cells, and the majority of these are idiotype related and found only on T-and B-cell tumors. Thus, for many tumors, vaccination strategies must induce CTLs that are able to recognize internally expressed antigens. This approach can be especially effective in cases in which the tumor itself fails to fulfill the requirements for antigen presentation or fails to elicit an immune response by cross-priming of professional APCs. To elicit CTL responses *in vivo*, either the requisite antigens must be expressed by professional APCs, or the tumor cell itself must be transformed into an APC. The strategy of choice depends in part on whether the sequence of the tumor antigen is known, whether CTL epitopes have been mapped, and whether tumor material is available and amenable to genetic modification.

#### *DNA Vaccines*

When tumor antigens have been identified and cloned, naked DNA vaccines injected directly into skin, muscle, or mucosal surfaces have proved capable of inducing both CTL and antibody responses. The type of response depends on the route of immunization, the immunogen, and the species immunized.[242] Most of the immune response to DNA vaccines is thought to result from expression of antigen by nonlymphoid tissues and subsequent transfer to and cross-presentation by professional APCs. However, direct antigen presentation by transduced nonlymphoid cells and direct transduction of APCs themselves may also occur.[243] The advantage of DNA vaccines is that they are stable and inexpensive and relatively simple to administer. Furthermore, the type of immune response elicited may be manipulated by coinnoculation with cytokines, cytokine DNA, or immunostimulatory molecules, such as CD80 or CD40L.[244,245] The potential disadvantages are that because the DNA is expressed long term in normal cells, the immunogen must be safe (it should lack oncogenic potential, in particular), the recipient must have a competent immune system to be able to respond to the immunogen, and the tumor antigen must have been cloned and characterized. Melanoma, prostate CA, and virus-associated tumors are potential candidates for this strategy. There have as yet been few clinical trials of DNA vaccination, and most of the animal studies have focused on the treatment of infectious diseases. Nevertheless, DNA vaccination promises to be an important advance in the prevention of disease and the treatment of CA.[246] In addition, for self-antigens, autoimmunity is a possibility.

#### *Antigen- or Peptide-Pulsed Dendritic Cells*

### Expressing Antigens in Dendritic Cells

One way to ensure that an immunogen is presented in the optimal way is to infuse APCs that have been generated and “loaded” with antigen *in vitro.* Because DCs are the most potent APCs and are capable of inducing primary immune responses and overcoming immune tolerance, they have been used in many preclinical and clinical studies to induce immune responses to tumors. They can be generated from precursors in peripheral blood or bone marrow by culture in cytokine cocktails, most commonly IL-4 and GM-CSF.[247] Between 1% and 3% of PBMCs can differentiate into DCs, and although this percentage can be dramatically increased by G-CSF mobilization, only $CD11c^+$ DC precursors increase in numbers in response to G-CSF, and this subset has been reported to stimulate Th2 CD4 T cells preferentially.[248] Activated DCs efficiently phagocytose and process dying cells and particulate matter, but for efficient activation of T cells, they need an additional maturation step. This can be achieved *in vitro* by the addition of TNF-a or cytokine cocktails or by engagement of the CD40 or Fc receptors on the DC surface.[249,250] and [251] DCs can be loaded with antigen in many ways. The simplest method, if adequate CTL epitopes have been identified, is to incubate the DCs with the peptide epitopes, which then are presented in association with MHC molecules at the cell surface. Use of peptides is limited by the HLA phenotype of the patient, however, and if few target epitopes have been identified, the chances for immune evasion by mutation of the target antigen may be high. Furthermore, only a few of the class II helper epitopes that may be required to provide T-cell help have been identified. Whole protein loading could overcome these problems and ensure that the full repertoire of class I and class II peptides are available for any HLA phenotype.

Even with this strategy, methods are needed to direct the protein into the cytosol for class I processing. Osmotic lysis of pinosomes, liposome formulations, and Fc

receptor targeting have all been shown to allow protein targeting to the class I pathway, although mostly in murine systems. [252],[253] and [254] Antigen coupled to beads or to VLPs induces phagocytosis by professional APCs, resulting in MHC class I presentation. [255] A promising approach for introducing antigens into the cytosol of DCs is to use virus- or toxin-derived fusion proteins, which have endosomolytic activity resulting in the release of proteins into the cytosol. [256] For example, a fusogenic peptide derived from HIV-TAT effectively carries genetically linked proteins into the cytosol of almost any cell. [257],[258] There is now much interest in the generation of synthetic endosomolytic peptides for both protein and gene delivery. [259],[260] Alternatively, viral vectors can be used to introduce cloned antigens into DCs. The transgene is naturally translated in the cytosol and thus enters the class I processing pathways. Adeno- and lentiviral vectors can efficiently transduce and express transgenes in DCs at high multiplicities of infection. [261],[262] Retroviral vectors cannot transduce DCs but can transduce the $CD34^+$ precursors from which DCs can be derived if bone marrow or mobilized stem cells are available. [263] Additional advantages of viral vectors are that they can be used to express multiple antigens, which should reduce the incidence of escape mutations, and to improve the immunogenicity of the desired antigen by manipulating its structure. For example, when mice were vaccinated with DCs transduced with the retrovirus-encoded protein of hepatitis B virus, they generated only an antibody response. If the DNA was modified so that the encoded protein contained a secretory signal and an Fc receptor, then the protein was secreted and reinternalized by the DCs via the Fc receptor pathway *in vitro.* Mice immunized with DCs expressing this construct generated both CTL and antibody responses. [264] Target antigens have not been identified for most tumors. This limitation can be overcome by pulsing activated DCs with tumor lysates, peptides eluted from tumors, or apoptotic or necrotic tumor cells. Immature DCs possess unique pathways for the uptake and presentation of peptides and antigens from apoptotic and necrotic cells, and all of these antigen sources have been used successfully to generate $CD8^+$ CTLs *in vitro* and *in vivo.* Whole tumor extracts provide additional advantages in that they permit multiple tumor antigens to be presented, minimizing the chances for the evolution of CTL escape mutants, they overcome HLA phenotype restrictions, and they allow antigens to be presented on both class I and class II molecules.[265] Melanoma lysates were shown to be as effective as peptides in the induction of $CD8^+$ CTL responses from melanoma patients *in vitro* but less effective than apoptotic tumor cells. [266],[267] The most stringent requirement with these procedures is the need for fresh or cryopreserved tumor material.

### Clinical Trials with Dendritic Cell Vaccines

The majority of human clinical trials of DC therapy have tested peptide or tumor lysate–modified DCs in the treatment of melanoma and prostate CA. These tumors were chosen largely because they possess recognized tumor antigens that can elicit an immune response. Although these tumors are uncommon in children, the lessons learned from these diseases may be applicable to pediatric tumors. When GM-CSF–induced monocytes pulsed with a single melanoma-specific peptide were infused into patients, they produced CTL responses *in vivo* but no clinical responses.[268] Infusion of bona fide DCs pulsed with two HLA-A2–restricted prostate-specific peptides were more promising.[269] Thirty percent of the patients were partial responders, and 58% of these were still responsive at the end of the follow-up period. Mackenson and co-workers[270] reported on the treatment of 14 HLA-A1 or -A2 melanoma patients with pools of HLA-A1– and -A2–restricted peptides from the *MAGE-1*, *MAGE-3*, Melan-A, tyrosinase, and gp100 proteins, noting only mild toxic reactions, immunologic responses in four patients, and tumor responses in two. Nestle and co-workers[271],[272] used DCs pulsed with melanoma lysates or peptide cocktails (to diminish the chances of immune escape) selected according to HLA type to vaccinate 30 patients who had relapsed on standard protocols. All patients developed strong delayed-type hypersensitivity immune responses, which correlated with the eight clinical responses (27%), including three CRs. Tumor regression was noted in multiple tumor locations, even in patients with large tumor burdens, and toxicity was mild to moderate. Responses were not related to whether patients were treated with peptide or tumor lysate-pulsed DCs. The authors documented various immune escape mechanisms, including defects in the expression of proteasomal enzymes, TAP deficiency, antigenic loss variants, and down-regulation of HLA expression, commenting that these factors should be considered limitations of therapy. Additional escape mechanisms were identified in studies of melanoma-specific CTL precursor frequencies in patients with metastatic melanoma.[273] In one study, circulating CTLs were identified in 6 of 11 patients, and in one of these, 2.2% of the T cells were specific for a single tyrosinase peptide. This population was isolated and shown to be functionally unresponsive. [274] Strategies to overcome immune escape mechanisms are under consideration, including the combination of peptide-pulsed DCs with systemic recombinant IL-2 to enable DC-activated CTLs to expand and function *in vivo*.[275] One caveat concerning the use of DCs with other immunostimulatory molecules to "superactivate" the immune response is that it may break immunologic tolerance to self-antigens and produce autoimmunity. This risk is well illustrated by our experience with the lethal murine B-lymphoma A20 cell line, which failed to induce an immune response in syngeneic mice. If the mice were first vaccinated with syngeneic DCs pulsed with peptides eluted from the tumor in combination with syngeneic fibroblasts engineered to express CD40 ligand and IL-2, however, they were protected against lethal doses of the tumor but developed autoimmune disease.[276] A similar although not life-threatening response was observed in melanoma patients, who developed vitiligo (depigmented skin) after vaccination with melanoma peptide–pulsed DCs.[270]

## *Tumor Vaccines*

### Generating Immunogenic Tumors

An alternative strategy, when tumor antigens have not been identified, is to genetically modify the tumor cells themselves to improve their ability to induce an immune response. This strategy, termed *tumor vaccination*, has many variations. Tumors can be modified either *in vivo* or *ex vivo* with a range of gene transfer methods, including retroviral and adenoviral vectors, lipofection, or bioballistic techniques. [277] The advantage of *ex vivo* modification is that it permits early evaluation of transgene expression, control of the number of modified tumor cells infused, and escalation of the vaccine dose. *Ex vivo* modification is particularly appropriate for tumors such as neuroblastoma that can be expanded in tissue culture.[278] A potential disadvantage is that the tumor cells growing in culture may not represent the tumor cells that grow *in vivo.* Modification of tumors *in vivo* is more difficult to control, because it is impossible to determine the number of tumor cells that have been modified, and this end point varies from patient to patient, even when the same dose of viral vector is used. Nevertheless, small numbers of tumor cells modified *in vivo* should be able to induce a specific, systemic immune response that can target unmodified tumors at distant sites.

Tumors can be modified to improve one or multiple phases of CTL activation and expansion ( Table 14-2). The first phase is the attraction and activation of professional APCs, for which GM-CSF and CD40 ligand are the preferred molecules. The second phase is the recruitment of T cells with use of chemokines such as lymphotactin.[279] The third phase, T-cell activation, has been achieved by modifying tumor cells with B7 and CD40 to improve antigen presentation. IL-2 and IL-12 have been used to provide T-cell help to secure the fourth phase, T-cell expansion. In the fifth (or effector) phase, activated CTLs may be anergized or killed by factors released by the tumor or even killed directly by tumor cells. For example, tumor cells expressing FAS ligand can trigger CTL apoptosis by engaging the death receptor, FAS, on CTLs.[280] Influencing this phase may not be a realistic goal of tumor vaccine studies, but the possibility is addressed later in the section Antigen-Specific Cytotoxic T Cell. Although most tumor vaccine strategies are directed at one or two phases of immune activation, improving one phase usually improves other phases as well. GM-CSF affords a particularly good example of this principle. Tumors expressing recombinant GM-CSF recruit and activate macrophages and DCs, which in turn secrete inflammatory cytokines that recruit T cells. The activated DCs can then phagocytose dying tumor cells and activate the recruited $CD4^+$ and $CD8^+$ T cells. The $CD4^+$ T cells produce IL-2 and help CTL expansion. So although used primarily to influence the first phase of CTL activation and expansion, GM-CSF also acts on the three subsequent phases. This may explain why GM-CSF has been the most effective cytokine for boosting immune responses to tumors.

| Step | Function | Ligand | Cytokine | Inhibitory factor |
|---|---|---|---|---|
| 1 | Activation of antigen-presenting cells | Dying cells, viruses | Granulocyte-macrophage colony-stimulating factor, IL-4, tumor necrosis factor-α | IL-10, TGF-β |
| 2 | Attraction of T cells (T helper 1 type) | Costimulatory molecules | Lymphotactin, chemokine ligand 2, macrophage inflammatory protein 1-α,β | Thymus and activation-regulated chemokine |
| 3 | T-cell activation | Major histocompatibility complex-peptide, CD86, CD40 | Interferon-γ | TGF-β |
| 4 | T-cell expansion | – | IL-2, IL-12, IL-7 | TGF-β |
| 5 | Tumor cell killing | – | IL-10 | FAS ligand |

IL, interleukin; TGF-β, transforming growth factor β.

**TABLE 14-2. MOBILIZATION OF THE CELLULAR IMMUNE RESPONSE TO TUMORS**

### Preclinical Studies of Tumor Vaccines

The first studies of tumor vaccines showed, in a murine model, that influenza virus–infected tumors could elicit enhanced immune responses to uninfected tumors. [281]

This outcome likely resulted partly from the virus-mediated induction of an inflammatory response and partly from the assistance of T helper cells specific for strong influenza epitopes. Similarly, the introduction of allogeneic MHC molecules boosted immune responses to tumors, probably because of inflammation caused by allostimulation and subsequent stimulation of infiltrating tumor-specific T-cell precursors. Autologous MHC class II molecules have also been used to stimulate help for MHC class II–negative murine tumors.[282] This strategy proved successful and could be improved by comodification with costimulatory molecules, such as B7, superantigens, and cytokines.[283] For example, mice with established metastatic mammary tumors responded to vaccination with tumor cells modified with B7 plus MHC class II antigens, injected together with IL-12. This combination was far superior to any one or two modifications alone. However, a major limitation to the systemic use of cytokines has been their toxicity. IL-2, IL-12, and GM-CSF are highly toxic if given in concentrations strong enough to produce local effects, although high local concentrations of cytokines with only slight systemic toxicity can be achieved by engineering tumor cells to secrete the cytokines. [284] If tumor cells are unavailable or are unamenable to gene transfer, autologous fibroblasts modified to express cytokines can be substituted. [285] There is a wealth of data in animal models to show that IL-4, IL-7, IFN-g, and TNF-a can also be used to improve tumor-specific immunity.[286] The results of vaccination with tumor cells depend on a host of interacting factors, including tumor type, type of immune evasion strategy used by the tumor (usually not known), vaccine dose, level of transgene expression, challenge site, and vaccine schedule. Hence, it has been difficult to identify a “best strategy” for translation into human trials.

### Clinical Studies

When used in patients, tumor vaccines have produced little systemic toxicity, with only local inflammation and sometimes patches of vitiligo in melanoma patients. [287] In human clinical trials, tumors have been modified with IL-2, IL-4, IL-7, IFN-g, and GM-CSF. Infusion of unmodified melanoma cells mixed with IL-2–expressing autologous fibroblasts resulted in infiltration of $CD4^+$ and $CD8^+$ T cells, which produced tumor cell lysis after culture *in vitro.* Some mixed responses and disease stabilization were seen in approximately 10% of patients. Autologous neuroblastoma cells expanded *in vitro* and transduced with an adenoviral vector expressing IL-2 were infused into children with advanced neuroblastoma.[288] Of ten children, one had a CR, one had a partial response, and three had stable disease. Four of five responders had an increased frequency of tumor-specific CTLs, compared with only one of five children who did not have a tumor response. Neuroblastoma cells cannot be cultured to large numbers in every case. Therefore, because DCs can phagocytose tumor cells and present shared antigens in association with their own MHC molecules by cross-priming, allogeneic HLA-A2–matched IL-2–transduced neuroblastomas were also used to vaccinate 15 children from whom autologous tumors could not be grown. Although the responses were not as good as those obtained with autologous tumors, one child had a partial response and seven had stable disease.[289] The authors suggested that combinations of cytokines and costimulatory molecules are likely necessary to improve response rates, and such studies are planned. Cross-priming with allogeneic tumors can also produce alloimmunity, but this reaction should not pose problems except in recipients of organ or marrow transplants. Hematopoietic tumors have been less amenable to the tumor vaccine approach because of their poor transduction rates with currently available, clinically acceptable vectors. Disabled herpes simplex virus vectors can efficiently transduce a wide range of cell types, including hematopoietic cells, but they have not yet been used in clinical trials. [281] An interesting combination of tumor and DC vaccination was recently tested in 17 patients with advanced renal cell carcinoma. After immunization with hybrids of autologous tumor and allogeneic DCs generated by electrofusion, four patients completely rejected all metastatic lesions, one had a mixed response, and two had a greater than 50% reduction in tumor mass.[290]

### *Ex Vivo* Induction of Cytotoxic Lymphocytes for Clinical Use

*Ex vivo* expansion of tumor-specific killer cells has been evaluated with the intent of overwhelming tumor cells by infusing large numbers of killer cells. Such adoptive transfer strategies have used LAK cells, TILs, donor leukocytes, and tumor-specific CTL lines and clones.

#### *Lymphokine-Activated Killer Cell Therapy*

LAK cells are NK cells that have been activated and expanded in IL-2–containing cultures. They have HLA-unrestricted cytotoxic activity against a range of human tumors *in vitro*, but their role *in vivo* is unclear. In the first clinical applications, LAK cells were infused with IL-2 into patients with advanced renal carcinoma and melanoma, resulting in some clinical responses, including a complete response in a patient with metastatic melanoma. [291] LAK cells with IL-2 were subsequently compared with IL-2 alone in patients with end-stage CA.[292],[293] and [294] Although results of these initial studies were disappointing, the potential of this combination is being explored further in the treatment of early or minimal residual disease, as adjuvant therapy to prevent leukemic relapse after stem cell transplantation, or after activation with other combinations of cytokines.[295] Because LAK cells have not produced satisfactory response rates, they have been replaced whenever possible by TILs, with the thought that these cell populations would contain tumor antigen–specific CTLs.

#### *Tumor Infiltrating Lymphocyte Therapy*

Many tumors contain “infiltrating T cells,” whose presence is associated with the regression of melanoma and cervical carcinoma, to cite two examples. If these T cells could be expanded *in vivo*, they might produce greater antitumor responses; however, not all tumors possess TILs, and such cells have often proved difficult to extract and grow. TILs may be extracted by needle biopsy, but then must be expanded to greater than or equal to $1 \times 10^{11}$ for therapeutic use.[296] This *ex vivo* expansion may result in the overgrowth of nonspecific cells because of the lack of specific antigen stimulation. For these reasons, TIL therapy has been restricted in part by the tumor type and location. $CD8^+$ TILs that specifically recognize autologous tumor tissue can be grown from 50% of melanomas and have been used to identify more than 30 new melanoma-specific peptides.[297],[298] and [299] Tumor-specific $CD4^+$ TILs have also been grown from melanomas and from breast, ovarian, and colon CAs.[296],[300] Such cells may exert cytotoxic action either directly or by releasing cytokines, such as TNF-a, GM-CSF, and IFN-g, in response to the autologous tumor in a class II–restricted fashion.

The presence of tumor-specific CTLs in actively growing tumors raises the question of whether they play any role in tumor regression. Evidence to support an antitumor function of TILs comes from the association of these cells with a better prognosis and the tendency of TIL-containing tumors to undergo spontaneous regression.[301] Tumor progression may in fact result from the anergization of tumor-specific CTLs *in situ* by tumor antigen expression in the absence of costimulatory molecules or by inhibitory cytokines released by the tumor cells. By activating and expanding these specific cells *in vitro* in the absence of inhibitory signals, it may be possible to restore their function in culture so that they remain active and able to produce antitumor effects when returned to the patient. Although responses to TIL therapy have been better than those to LAK cells, the response rates are still low, and toxicity, although less severe than with IL-2, remains clinically significant. Current efforts are attempting to improve the efficacy, persistence, and specificity of TIL infusions by genetic modification of cytokine production and expression of tumor-specific single-chain antibodies. Finally, tumor-specific CTLs can be isolated from the TIL population and specifically expanded for use as CTL therapy.

#### *Donor Leukocyte Infusions*

The curative effects produced by allogeneic HSCT for malignant disease entail not only high-dose chemotherapy and radiotherapy administered as bone marrow conditioning but also GVL reactions. The presence of GVL was originally suggested by the higher relapse rates in recipients of syngeneic or T-cell–depleted transplants and lower relapse rates in patients who developed GVHD. [302] These immune-mediated antileukemic effects are probably mediated by several different mechanisms, including recognition of alloantigens such as major or minor histocompatibility complex molecules or tumor-specific antigens expressed by tumor cells.

Adoptive immunotherapy with DLIs provides a means of augmenting the GVL response after HSCT to eliminate residual disease. In 1990, Kolb and co-workers [303] reported on three patients with relapsed CML who attained complete cytogenetic remissions after treatment with IFN-a and DLI. In larger series, approximately 70% of all relapsed CML patients treated in the chronic phase achieved complete cytogenetic remission, in contrast to only 11% of those in the accelerated phase or blast crisis.[304] For patients with other hematologic malignancies relapsing after transplant therapy, DLI has resulted in a much lower response rate: only 29% of patients with AML and 5% of these with ALL.[305]

DLI is associated with toxicity and treatment-related complications that include GVHD and pancytopenia. [306] Attempts have been made to separate the GVL effect from unwanted alloreactivity by administering either smaller doses of cells or subsets of lymphocyte. In one series in which the risk of alloreactivity was reduced by administering smaller doses of cells, some patients who received $1 \times 10^7$ $CD3^+$ cells per kg attained remission without developing GVHD. [306] Because of varying degrees of alloreactivity with different donor-recipient pairs, however, it is probably not possible to identify a dose that always produces GVL without GVHD. When lymphocyte subsets are used as an alternative approach, either CD8-depleted or CD4-selected cells can induce remission in CML patients with only a low incidence of GVHD.[307],[308] Immunostimulatory cytokines, such as IL-2, may amplify GVL mechanisms and induce remissions in patients who have failed to respond to DLIs. Slavin and co-workers,[309] using DLI with or without IL-2–activated cells for remission induction after leukemic relapse after HSCT, reported CR in 10 of 17 patients, nine of whom responded only after donor cell activation with IL-2. [309] A shorter median time to CR has also been described with the addition of IFN-a to DLI in the

treatment of relapsed CML after allogeneic HSCT.[310]

### *Antigen-Specific Cytotoxic T Cells*

#### Cytotoxic T-Cell Generation

Although vaccination strategies can overcome poor tumor-antigen presentation, they are unlikely to overcome the problem of tumor-mediated inhibition of CTL expansion. Thus, although CTLs can be activated by vaccination, they may not expand *in vivo.* One way to resolve this problem is to infuse tumor-specific CTLs that have been activated and expanded *in vitro.* Expansion of CTLs *in vitro* provides additional advantages: CTL reactivity with tumor and normal target cells can be analyzed before the cells are infused, and the CTL dose can be escalated in a controlled fashion. Thus, infusion of auto- or alloreactive CTLs can be avoided, an important consideration in stem cell recipients requiring DLI for leukemic relapse.

The activation of CTLs *in vitro* requires APCs expressing only the antigens that will stimulate the desired CTLs without activating unwanted T cells, which might overgrow the culture or possess auto- or alloreactivity. Similarly, neither the culture medium and growth factors selected for expansion of CTLs nor the antigens used for restimulation should expand unwanted T cells. This remains a major challenge, especially when the CTL precursor frequency is low or the tumor antigens weak (Fig. 14-8). As for the activation of CTLs *in vivo,* DCs modified with peptides, proteins, RNA, or viral transgenes have been used to stimulate secondary and, occasionally, primary CTL responses.[311,312 and 313] Again, if tumor antigens have not been identified and tumor material is available, DCs pulsed with apoptotic or necrotic tumor cells or extracts can be used to reactivate tumor-specific CTLs.[226] DCs can also overcome immunologic tolerance to self-antigens expressed on tumor cells, provided they express high levels of antigen. For example, PSA-specific CTLs were generated from healthy volunteers by stimulation with autologous DCs transfected with messenger RNA encoding PSA.[314] The danger of this approach is that if tolerance to self is broken, then autoimmunity may be induced.[276] Thus, when using a “self” antigen as a tumor target, it is important to know its tissue distribution. Although CTLs can be tested for autoreactivity against normal target cells before infusion, few tissue types are usually available as targets.

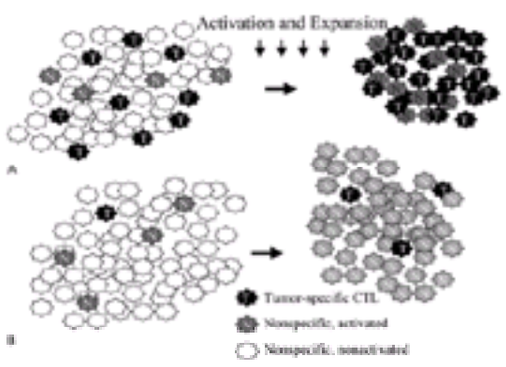


**FIGURE 14-8.** Bystander activation can overwhelm the cytotoxic T-cell (CTL) response to specific antigen. **A:** When there is a high frequency of tumor-specific CTL precursors, the coexpansion of small numbers of bystander cells is irrelevant and does not inhibit CTL function. **B:** Conversely, in a culture in which the CTL precursor frequency is vanishingly small, then the same number of bystander cells can overwhelm the culture so that CTL function cannot be detected.

Less has been published about ways to substantially expand activated CTLs *in vitro* without losing specificity. It may not be feasible to restimulate CTLs repeatedly with DCs owing to the large amounts of blood required for DC generation. Furthermore, although DCs efficiently activate T cells, they do not provide good help for expansion (unpublished observation). Nonspecific activation with stimulatory antibodies to CD3 and CD28 in the presence of allogeneic PBMC feeders is effective for CTL clones but specificity can be lost when this method is applied to polyclonal cultures.[315]

The generation of EBV-specific CTLs for adoptive therapy has been relatively simple because most individuals are persistently infected with this virus and have a high prevalence of EBV-specific CTL precursors, which can readily be reactivated and expanded *in vitro* by coculture with an autologous EBV-transformed B-cell line (LCL).[316] If bystander cells are activated, they represent only a small proportion of the culture. LCLs are excellent APCs, as they can be generated from any normal individual as well as from most patients, and they provide an infinite source of APCs cells and target cells against which to test CTL function.[317] Although we have been able to coinduce responses to EBV and adenovirus (both strong recall immunogens), using adenovirus-pulsed LCLs, LCL cannot be used to stimulate responses to weak antigens or to reactivate low-frequency CTLs because the strong antigens are likely to dominate the response.[318]

#### Immunotherapy with Antigen-Specific Cytotoxic T Cells

Riddell and co-workers[238] pioneered the use of antigen-specific CTLs to prevent CMV reactivation in marrow recipients. Donor-derived $CD8^+$ CTL clones activated by coculture with CMV-infected autologous fibroblasts and specific for the viral tegument proteins pp65 and pp150 proved safe and protected recipients of T cell–replete HSCT against the reactivation of CMV. However, the persistence of the $CD8^+$ clones was dependent on the recovery of endogenous $CD4^+$ CMV–specific T cells.[315] Later studies showed that coinfusion of $CD4^+$ clones with the $CD8^+$ clones was sufficient to ensure persistence of the latter.

We have used donor-derived, polyclonal CTL lines to prevent and treat EBV-LPD in children receiving T cell–depleted stem cell transplants. These CTLs were genetically marked with a retroviral vector so that their fate *in vivo* could be determined. The CTLs expanded several logs *in vivo* after infusion and then persisted for up to 7 years,[239,241] most likely because of the presence of both $CD4^+$ and $CD8^+$ T cells in the lines, the continued presence of EBV, and the fact that the CTLs were infused into an empty niche in a regenerating immune system. The infused CTLs were safe and effective, and all patients who received prophylactic CTLs were protected from EBV-LPD. This outcome contrasts with the 11.5% of children who did not receive CTLs and developed posttransplantation lymphoproliferative disease.[241] The infused CTLs rapidly and permanently reconstituted immune responses to EBV and reduced the virus load to the point at which EBV-LPD became likely. EBV-specific CTLs were also effective in three of four patients with bulky disease who received CTL therapy. A single patient who did not respond illustrates one of the recurring problems of immunotherapy: mutation of CTL target epitopes on tumor cells leading to the evasion of cytolysis. This patient had two types of circulating viruses at the time of diagnosis. One had a deletion in the *EBNA3B* gene of EBV that removed two immunodominant HLA-A11–restricted CTL epitopes. The major cytolytic activity of the CTL line from the HLA-A11–positive donor was directed at the two deleted epitopes. After CTL infusion, the virus with wild-type *EBNA3B* disappeared from the patient's circulation, whereas the virus with the deletion persisted.[105] Thus, the infused CTL line selected for an escape mutant. That a CTL escape mutant could occur in a virus with a remarkably stable genome indicates that the problem may be much greater in tumors, whose genomes are generally more unstable. Indeed, as mentioned previously, CTL escape mutants have also been described in melanoma.[319,320] To avoid this phenomenon, it will clearly be necessary to target multiple epitopes, preferably on essential proteins that cannot be down-modulated.

EBV-specific CTLs have also proved effective in an autologous setting in solid organ recipients and in a patient with DiGeorge syndrome.[319,320] Thus, even in severely immunocompromised individuals whose CTLs cannot effectively control EBV *in vivo*, CTLs can be reactivated and expanded in culture and returned in sufficient numbers to control the underlying disease. It should be emphasized that EBV-associated tumors arising in severely immunocompromised individuals require only minimal immune evasion strategies, as they are highly immunogenic and are readily eliminated by normal CTLs. By contrast, immunogenic tumors that arise in immunocompetent individuals rely on multiple immune evasion strategies, posing a much greater obstacle to treatment with infused CTLs. For example, the EBV-carrying RS tumor cells in HD express viral target antigens, high levels of MHC class I and class II, and costimulatory molecules, and have an intact antigen-presenting machinery. Furthermore, patients with HD have a normal frequency of circulating EBV-specific CTLs and control their normal EBV-infected B cells effectively. However, the RS cells secrete a variety of inhibitory chemokines and cytokines, including IL-10, TGF-b, and TARC, that function locally in the tumor environment and inhibit EBV-specific CTL function (Fig. 14-9A and Fig. 14-9B).[321,322] IL-10 is a “schizophrenic” cytokine with both Th1 and Th2 effects depending on the target cell.[323,324,325 and 326] Its function in Hodgkin's tumors is likely to deactivate local professional APCs by down-regulating their expression of class II molecules and inhibiting their release of inflammatory cytokines. This, in turn, will prevent cross-priming of Hodgkin tumor antigens, including EBV antigens, by APCs and the resultant recruitment of T cells. TGF-b also has pleiotropic effects depending on the target cell type, but it inhibits the development of CTL responses via early signaling pathways and can directly induce apoptosis in some cell types.[232,327] TARC is a chemokine that specifically recruits $CD4^+$ Th2 cells that can contribute to a pro-Th2 and anti-Th1 environment. In addition, RS cells express FAS ligand, a death receptor that may induce apoptosis in FAS expressing $CD8^+$ CTLs (Fig.

14-9B).[328] Thus, Hodgkin's tumors protect themselves against CTL lysis by relying on several mechanisms, some or all of which must be circumvented in any adoptive transfer strategy.

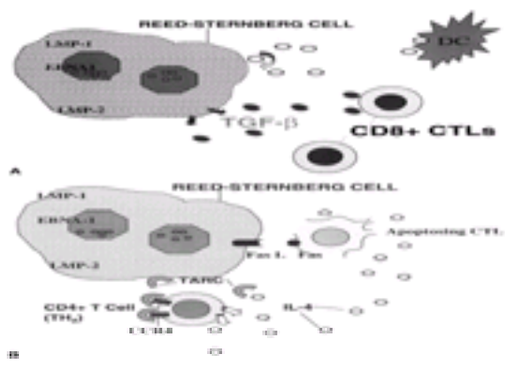


**FIGURE 14-9.** Multiple immune evasion strategies used by Hodgkin Reed-Sternberg (H-RS) cells. **A:** The H-RS cells express only a subset of the viral latency-associated proteins, LMP1 and 2 and EBNA1. The immunodominant EBNA3 proteins are not expressed. The tumor cells also secrete interleukin 10 (IL-10), which may prevent the maturation of local professional antigen-presenting cells by down-regulating major histocompatibility complex (MHC) class II expression and preventing the release of inflammatory cytokines. Thus, cross-priming of tumor antigens is inhibited. The tumor cells also secrete transforming growth factor b (TGF-b), which prevents T-cell activation and inhibits the release of interferon-g in response to IL-12. **B:** H-RS cells release thymus- and activation-regulated chemokine (TARC), a chemokine that recruits T helper 2 (Th2) cells, which secrete IL-4 and help antibody responses while inhibiting cytotoxic T cell (CTL) responses. Finally, H-RS cells express Fas ligand, which may induce apoptosis in Fas-expressing CTLs that may penetrate the other immune evasion strategies and make contact.

Despite these potential obstacles, we have used gene-marked autologous EBV-specific CTLs to treat patients with multiply relapsed EBV-positive HD. Five patients with aggressive disease at the time of CTL infusion survived for 3 to 12 months, and three patients are still alive 6 to 14 months after infusion. Three of four patients had resolution of B symptoms after infusion, and four had a mixed response, including tumor responses at some sites but not others. The two patients who received CTLs after autologous transplants remain in remission for 4 and 5 months postinfusion.[329] These studies demonstrate that the infused CTLs had antiviral effects (e.g., reduction in virus load), improved the CTL precursor frequency, and persisted for up to 5 months after infusion. Furthermore, gene-marked CTLs homed to malignant sites. Nevertheless, none of the patients was cured, clearly indicating a need for improvements through strategies based on our current knowledge of the tumor's immune evasion tactics described earlier. Successful treatment may ultimately require CTLs made resistant to some or all of the inhibitory factors by genetic modification.

Leukemia-specific antigens have been identified as CTL targets in patients undergoing allogeneic HSCT for leukemic relapse. These include minor antigens differentially expressed on hematopoietic cells or lineage-specific antigens, such as WT1 or proteinase 3, that are selectively expressed in malignant cells.[262,330,331] The minor histocompatibility antigens HA1 and HA2 induce HLA-A*0201–restricted CTLs *in vivo* and are selectively expressed on hematopoietic cells, including leukemic cells and their precursors. Mutis and co-workers[332] investigated the feasibility of *ex vivo* generation of HA1- and HA2-specific CTLs from HA1- or HA2-negative donors, using peptide-pulsed DC cells as APCs.[333] The resulting HA1- and HA2-specific CTLs lysed leukemic but not nonhematopoietic cells from patients with AML or ALL. Such lines could therefore potentially mediate cytotoxic activity directed at recipient hematopoiesis (and leukemia) but not at donor hematopoiesis, when the donor was HA1 or HA2 negative and the recipient HA1 or HA2 positive. Two recent reports have investigated the use of the WT1 antigen, which is expressed at higher levels in acute and CMLs than on normal hematopoietic progenitors.[330,333] WT1-specific CTLs killed leukemic cell lines and selectively inhibited the growth of leukemic colonies, suggesting the potential of this reagent for immunotherapy. Another differentiation antigen overexpressed in tumor cells is proteinase 3, found in high concentrations in granules of malignant myeloid blasts. A recent report demonstrated the feasibility of selecting CTLs that recognize the PR1 peptide derived from this protein using HLA-peptide tetramers.[331] Expanded lines selectively lysed CML blasts cells.

## FUTURE PERSPECTIVES

There are now many exciting bioengineering strategies and animal models that demonstrate how the immune system can be beneficially manipulated with immunoreactive molecules. However, such models cannot always predict the outcome of immunotherapy for human tumors, so clinical testing of emerging concepts will likely be a major preoccupation during the coming years. It is important to understand why tumors evade immune responses, because only by understanding these mechanisms will we be able to develop strategies to overcome them. Immunotherapy for widespread or bulky CA will likely be most effective when used in combination with standard treatments, although in cases in which it can be used alone, it offers the possibility of sparing children major short- or long-term toxicities. Establishing the efficacy of this new modality at the time of minimal disease requires novel methods of evaluation, because randomized clinical trials are often impractical for rare CAs such as pediatric malignancies. The feasibility and cost of immunotherapy is frequently questioned. With few exceptions, most immunotherapies require sophisticated *in vitro* manipulations, some demanding pharmaceutical-grade “clean facilities” as well as experienced technical staff. Nonetheless, in view of the current cost of acute and late effects of cytotoxic therapy, continued effort to improve immunotherapeutic strategies seems highly worthwhile.

## ABBREVIATIONS

| | |
|---|---|
| ABMT | Autologous bone marrow transplantation |
| ADCC | Antibody-dependent cell-mediated cytotoxicity |
| AIDS | Acquired immunodeficiency syndrome |
| ALL | Acute lymphocytic leukemia |
| AML | Acute myeloid leukemia |
| APC | Antigen-presenting cell |
| BCG | Bacillus Calmette-Guérin |
| CA | Cancer |
| CDR | Complementarity-determining region |
| CEA | Carcinoembryonic antigen |
| CMC | Complement-mediated cytotoxicity |
| CML | Chronic myeloid leukemia |
| CMV | Cytomegalovirus |
| CR | Complete remission |
| CTL | Cytotoxic T lymphocyte |
| $^{67}$Cu | Copper 67 |
| DAF | Decay accelerating factor |
| DC | Dendritic cell |
| DLI | Donor leukocyte infusion |
| EBNA | Epstein-Barr virus nuclear antigen |
| EBV | Epstein-Barr virus |
| EBV-LPD | EBV-associated lymphoproliferative disease |
| EGF | Epidermal growth factor |
| G-CSF | Granulocyte colony-stimulating factor |
| GM-CSF | Granulocyte-macrophage colony-stimulating factor |
| GVHD | Graft-versus-host disease |
| GVL | Graft-versus-leukemia |
| HAMA | Human antimouse antibody |
| HD | Hodgkin's disease |

| | |
|---|---|
| HIV | Human immunodeficiency virus |
| HLA | Human leukocyte antigen |
| HSCT | Hematopoietic stem cell transplant |
| ICAM-1 | Intercellular adhesion molecule-1 |
| IFN | Interferon |
| Ig | Immunoglobulin |
| IL | Interleukin |
| LAK | Lymphokine-activated killer |
| LCL | Lymphoblastoid cell line |
| LFA | Leukocyte function antigen |
| M-CSF | Macrophage colony-stimulating factor |
| MDR | Multidrug resistance |
| MHC | Major histocompatibility complex |
| MoAb | Monoclonal antibody |
| MRP | Multidrug resistance protein |
| NK | Natural killer |
| PBMC | Peripheral blood mononuclear cell |
| PMN | Polymorphonuclear neutrophil |
| PR | Partial remission |
| PSA | Prostate-specific antigen |
| RES | Reticuloendothelial system |
| RIT | Radioimmunotherapy |
| RS | Reed-Sternberg |
| RTA | Ricin toxin A-chain |
| TAP | Transporter-associated protein |
| TARC | Thymus- and activation-regulated chemokine |
| TCR | T-cell receptor |
| TF | Thomsen-Friedenreich antigen |
| Th1 | T helper 1 (pro-CTL) |
| Th2 | T helper 2 (pro-antibody) |
| TIL | Tumor-infiltrating lymphocyte |
| TNF | Tumor necrosis factor |
| VEGF | Vascular endothelial growth factor |
| $V_H$ | Variable region of Ig heavy chain |
| $V_L$ | Variable region of Ig light chain |
| VLP | Viruslike particle |
| $^{90}Y$ | Yttrium 90 |

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# 15

# INFANTS AND ADOLESCENTS WITH CANCER: SPECIAL CONSIDERATIONS

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## INTRODUCTION

The diagnosis and management of cancer in children at the extremes of the pediatric age group pose significant challenges to care providers and clinical investigators. The care of the infant with cancer is particularly challenging due to the exceptional vulnerability to the acute complications associated with aggressive, multimodal therapy and to the potential long-term sequelae of antineoplastic therapy on growth and development.

Cancer in the first year of life is relatively rare. [1,2] Infants with cancer often have a different clinical presentation from older children with the same disease, and their response to therapy also differs, indicating unique biologic properties of cancer in infants that explain the different clinical outcomes. [3,4 and 5] Although it is rare, cancer in the newborn and young infant has the potential to provide important insights into early human developmental oncobiology and suggests an intimate relation between oncogenesis and teratogenesis.[6]

The effect of very young age on prognosis and therapy depends on the specific diagnosis.

Cancer in the population of pediatric patients at the opposite extreme of age (i.e., adolescents and young adults) presents a completely different set of challenges. This group also has a distribution frequency of tumor types that differs from the general pediatric population. They have unique psychosocial and developmental issues, which must be sensitively and adequately addressed during therapy. Furthermore, access of adolescents to clinical trials and, therefore, to acceptable standard of care, is significantly inferior to the experience of younger children.

This chapter reviews the epidemiology of cancer in infants and adolescents and provides some guidelines for professionals faced with the challenge of treating these populations of patients. Also discussed are some of the unique biologic and clinical features of cancer in infants that have prognostic and therapeutic implications, as well as strategies to assure that adolescents and young adults gain access to appropriate multidisciplinary care and to improve their recruitment to clinical trials.

## CANCER IN INFANTS

### Epidemiology

Incidence data from the National Cancer Institute's (NCI) Surveillance Epidemiology and End Results (SEER) program indicate that the overall rate of cancer in U.S. children younger than 1 year of age is 218.4 cases per 1 million infants. [1] These data also suggest that the incidence is increasing ( Table 15-1).[2,7] The most common cancer in infants is neuroblastoma, followed by central nervous system (CNS) tumors, leukemia, retinoblastoma, renal tumors, germ cell tumors (including malignant teratomas), sarcomas, and hepatic tumors.[2] Unlike older children, female infants have a higher incidence of cancer than male infants, although rates for male infants are increasing.[2]

| Histology | 1970[a] | 1980[b] | 1990[b] |
|---|---|---|---|
| Neuroblastoma | 62.7 | 51 | 60 |
| Leukemia | 31.8 | 29 | 30 |
| Central nervous system | 14.0 | 24 | 33 |
| Retinoblastoma | 15.9 | 22 | 29 |
| Renal | 19.7 | 26 | 24 |
| Sarcoma | 17.8 | 10 | 11 |
| Germ cell | 0 | 5 | 6 |
| Teratoma | 2.8 | 4 | 13 |
| Hepatic | 7.5 | 4 | 8 |
| Lymphoma | 1.0 | 1 | 1 |
| Other | 9.3 | 13 | 5 |
| Total | 183.4 | 189 | 220 |

[a]Adapted from Bader JL, Miller RW. U.S cancer incidence and mortality in the first year of life. Am J Dis Child 1978;132:157. Incidence determined from the Third National Cancer Survey (10% sample of U.S. population) 1969 to 1971; rates per million live births per year in the United States.
[b]Adapted from Kenney LB, Miller RA, Reis LAG, et al. Increased incidence of cancer in U.S. infants, 1980 to 1990. Pediatr Res 1995; 37:159A. Incidence determined from Surveillance Epidemiology and End Results program of the National Cancer Institute (10% sample of U.S. populations) 1979 to 1988 and 1989 to 1991; rates per million children younger than 1 year of age in U.S. population 1980 and 1990.

**TABLE 15-1. INCIDENCE OF CANCER IN U.S. INFANTS**

As shown in Table 15-2, there is a difference in the percent distribution of tumor types in newborns and infants compared with all children younger than 15 years of age.[2,7,8] There have also been changes in the percent distribution over time. This change in percent distribution reflects stable rates of leukemia and renal tumors, and increased rates of CNS tumors and retinoblastoma.[2]

| | Children | Newborns | Infants |
|---|---|---|---|
| Histology | <15 yr (%) | <30 d (%) | <1 yr (%) |
| Leukemia | 31 | 13 | 14 |
| Central nervous system | 18 | 3 | 15 |
| Neuroblastoma | 8 | 54 | 27 |
| Lymphoma | 14 | 0.3 | 1 |
| Renal | 6 | 13 | 11 |
| Sarcoma | 11 | 11 | 5 |
| Hepatic | 1.3 | 0 | 3 |
| Teratoma | 0.4 | 0 | 6 |
| Retinoblastoma | 4 | 0 | 13 |
| Other | 6.3 | 5.7 | 5 |

**TABLE 15-2. PERCENT DISTRIBUTION OF THE MAJOR TYPES OF CANCER IN CHILDREN, NEWBORNS, AND INFANTS**

A report from the International Agency for Research on Cancer that compares population-based registry data from more than 50 nations demonstrates remarkable differences in international rates for cancer in infants. [9] Overall, the highest rates were found for Japanese infants, 252.81 cases per million child years, and the lowest were found for New York black infants, 109.28 cases per million child years. Israeli Jewish infants had the highest rate of neuroblastoma, and Swedish infants had the highest rate of retinoblastoma.[9,10] Comparison of incidence data from different nations has limitations, but it does serve to emphasize the relative contributions of genetics and environment to cancer in infants.

## Etiology

Although it is a rare event, cancer in infants presents a unique situation to study cancer etiology. In infants, the process of oncogenesis occurs in close temporal relation to embryogenesis. The time from the initiation of the malignant process to the clinical diagnosis of cancer in the infant is relatively short and easily delineated. Factors that should be considered as causes of cancer in infants include genetic susceptibility, acquired or constitutional; parental, intrauterine, and immediate postnatal environmental exposures; and transplacental metastasis.

Clinical evidence supports an inherited genetic susceptibility to developing cancer in infancy. For example, familial cases of Wilms' tumor and retinoblastoma occur at an earlier age than sporadic cases.[11,12] Moreover, some genetic syndromes are associated with cancer at an early age, such as Down syndrome with leukemia and familial adenomatous polyposis with infantile hepatoblastoma.[13,14] Genetic abnormalities have been identified that are frequently found in infants with cancer, such as chromosome band 11q23 breakpoint mutations (location of the MLL genes) in infant acute lymphoblastic leukemia (ALL), low N- *myc* oncogene copy in good-risk neuroblastoma in infants, and abnormalities in WT1 and the RB1 genes that are more commonly associated with the Wilms' tumors and retinoblastomas that occur in younger children.[11,15,16] and [17] A report on three pairs of infant twins with concordant leukemia and nonconstitutional gene rearrangements at 11q23 chromosome band breakpoint provides strong evidence for *in utero*–acquired genetic susceptibility to cancer. [18]

Taken together, this clinical and molecular evidence suggests that the cause of cancer in infants is related to an acquired or constitutional abnormality of cancer-predisposing genes that are critical during embryogenesis. The activation or suppression of these genes causes dysregulation of the normal developmental process and may lead to a malignant transformation in the infant. The fact that fetal and neonatal malignant tumors clinically manifested in the first few months of life can spontaneously regress or cytodifferentiate supports speculations about the physiologic expression of oncogenes by embryonal cells and their role in modulation of oncogenesis (see Chapter 3).[19]

Many studies have shown an association between parental exposure to environmental agents and cancer in the very young child ( Table 15-3).[20,21,22,23,24,25,26,27,28,29] and [30] Although these studies are not conclusive, they do suggest that some malignancies occurring in infants can result from preconceptional or *in utero* exposure of the developing fetus to environmental agents. Characteristics of the infant, such as preterm birth, have been associated with a relatively lower risk for neuroblastoma, but low birth weight in term infants is associated with increased risk. [31,32] These findings suggest a critical role for the timing of the environmental exposure during gestation and the consequent relation between teratogenesis and carcinogenesis.

| Cancer | Risk factor | Reference |
|---|---|---|
| ALL | Maternal history of prior fetal loss | 20 |
| | Paternal exposure to x-rays | 21 |
| | Maternal exposure to naturally occurring topoisomerase II inhibitors | 25, 26 |
| AML | Maternal use of marijuana | 22 |
| | Maternal exposure to topoisomerase II inhibitors | 25–30 |
| CNS (PNET) | Maternal diet deficient in fruits, vegetables, vitamin C, folate, nitrate | 23 |
| Hepatic | Maternal occupational exposure to metals, hydrocarbons, paints, and pigments | 24 |
| | Paternal occupational exposure to metals | 24 |

ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CNS, central nervous system; PNET, primitive neuroectodermal tumor.

**TABLE 15-3. PARENTAL FACTORS ASSOCIATED WITH INCREASED RISK OF CANCER IN INFANTS**

A documented, yet rare, cause of cancer that is unique to infants results from metastases of cancer in the mother to the fetus. Infant cases of leukemia and melanoma have been transplacentally acquired *in utero* from the mother.[33,34]

Studies that combine current knowledge of the unique genetic properties of cancer that occurs in infants with a focused epidemiologic investigation into specific environmental agents that could disrupt the normal expression or function of cancer-predisposing genes will be important in understanding oncogenesis and the cause of cancer in infants.

## Diagnosis

### *Symptoms and Signs*

Recognizing symptoms without having the benefit of subjective patient complaints presents a challenge. In young infants, particularly in newborns, the nonspecific findings of lethargy, somnolence, irritability, feeding difficulties, vomiting, fever or hypothermia, and failure to thrive could be caused by significant pathology, such as a malignancy. Although cancer is rarely the cause of these signs or symptoms in infants, it is still important to consider the possibility of this diagnosis.

There are findings on physical examination that should alert the examiner to the diagnosis of cancer in an infant. However, it may be difficult to differentiate benign from possibly malignant lesions, given the relative infrequency of cancer and the more common occurrence of developmental anomalies or nonmalignant conditions. Table 15-4 summarizes some clinical and laboratory abnormalities commonly associated with cancer and with the more frequent nonmalignant conditions observed in neonates and infants.

**TABLE 15-4. DIFFERENTIAL DIAGNOSIS OF MALIGNANT AND NONMALIGNANT CONDITIONS IN INFANCY**

### *Laboratory and Diagnostic Studies*

Laboratory techniques that require a minimal amount of whole blood, serum, and plasma have facilitated the diagnostic tests necessary for the evaluation of specific organ dysfunction and the existence of tumor markers. Radiographic investigations, including ultrasonography, computed tomography, magnetic resonance imaging, and radionuclide scans, are best performed in specialized pediatric centers, which provide technical and interpretive expertise in the diagnosis and management of infants and newborns (see Chapter 9). These radiographic studies guide the pediatric surgeon in determining the nature and extent of the operative procedure (i.e., biopsy or resection) required to establish a definitive diagnosis.

### *Pathologic Considerations*

The ultimate histopathologic diagnosis of cancer in infants requires the expertise of a pediatric pathologist. Specialized cytochemical, ultrastructural, and immunocytochemical techniques required to establish accurate diagnoses are discussed in Chapter 8. Although the pathologic findings of most tumor types are not unique to this age group, there are potential pitfalls in the pathologic diagnosis. For example, some tumors in infants can appear malignant microscopically but have a benign clinical course (e.g., infantile fibromatoses). [35,36]

## Management

### *Surgery*

The surgical management of the infant with cancer encompasses two major facets of care: fastidious attention to metabolic and physiologic details and adaptation of the extent of the surgical procedure to the unique biologic behavior of the specific tumor in this age group. Surgical care during the preoperative, operative, and postoperative periods must focus on temperature regulation; blood volume; fluid and electrolyte control (including calcium and phosphate); gestational development in the case of newborns; and the integrity of cardiac, pulmonary, and renal function. [37] Surgical care must be integrated into the overall treatment plan with regard to preoperative and postoperative chemotherapy and radiation therapy, which can alter nutritional status, wound healing, and immunologic function.

Maintenance of fluid and caloric intake is imperative. Feeding should be sustained as long as possible without interruption. If the infant is to be kept *non per os*, an intravenous glucose solution with maintenance sodium and potassium should be supplied. The rate of infusion should provide somewhat higher than maintenance fluid requirements to ensure good renal function, particularly if hyperosmolar contrast materials have been used for radiographic studies. Strict attention to the integrity of the coagulation system is also required in the young infant. Although vitamin K is routinely given at birth, additional doses may be needed to establish normal levels of vitamin K–dependent clotting factors.

In newborns, particularly those who are premature or small for gestational age, hypoglycemia, hypocalcemia, and environmental temperature must be observed and regulated. The infant's relatively thin skin, with a diminished layer of insulating subcutaneous fat, and the proportionately large surface area to weight ratio create a pronounced vulnerability to large heat losses. [37] Metabolic acidosis, vasoconstriction, and depleted plasma volume can result from increased metabolic rate induced by hypothermic stress. Temperature regulation requires avoidance of heat loss and may necessitate transporting infants on beds with overhead servocontrolled heating units.

In the operating room, the infant should be placed on a heated mattress, or heating lights should be used, and skin areas that can be covered should be wrapped. Another potential source of heat loss is the evaporated water from the respiratory tract. Effective temperature exchange from endotracheal ventilation during anesthesia can be used to control evaporative water and heat loss. A heated nebulizer should be used to saturate the ventilated gases with water vapor and to maintain the temperature of the inhaled gases. Intravenous fluids and transfused blood should also be warmed. [38]

Adequate venous access must be ensured. If significant pulmonary compromise or metabolic acidosis is apparent or anticipated or if significant blood loss is likely, an indwelling temporal, radial, or tibial artery cannula should be placed to monitor arterial blood gases and possible hypotension.

During the course of surgery, blood loss must be monitored closely. Sponges should not be wetted so that they can be weighed to estimate their blood content. The volume of blood loss from suction should be collected in a reservoir. The volume of “dead space” in the suction tubing between the patient and the calibrated measuring container should be minimized to provide greater accuracy of the blood loss estimate. [37] Hypocalcemia can occur as a result of the citrate in transfused blood, and effective levels of ionized calcium can also be lowered during administration of sodium bicarbonate to correct metabolic acidosis. [37] Prolonged operative procedures require an indwelling urinary catheter to monitor urinary output and to avoid overdistention of the bladder.

If large serosal surfaces, such as the thorax or abdomen, are exposed during the operation, a significant volume of serous fluid is sequestered in the third space. Replacement with 5% dextrose and Ringer's lactate approximates the composition of serum lost into the wound. The rate of infusion ranges from 5 to 15 mL per kg per hour, depending on the magnitude of loss. Rates of 150% to 200% of those used for maintenance fluids may be required. [37] The status of volume replacement postoperatively should be determined by assessing tissue perfusion. If there is concern, central venous pressure should be monitored. After major abdominal, thoracic, and neurosurgical procedures, most infants recover their preoperative status by the fifth postoperative day, with the resolution of ileus after abdominal surgery, return to oral feedings, and the beginning of wound healing. [37]

### *Radiation Therapy*

Radiation therapy plays a major role in the management of most pediatric cancers. Because of the potential for acute and chronic side effects, radiation must be used cautiously in infants. The severity of the side effects is assumed to be inversely related to the age of the child and directly related to dose. [39] Acute morbidity, such as gastrointestinal dysfunction, bone marrow suppression, and skin reactions, is seldom a limiting factor if radiation therapy is used alone, and the changes produced are usually reversible. However, pronounced late effects are not readily reversible. Scant published data exist on side effects unique to infants and newborns. Estimates of potential damaging effects generated from established specific tissue dose tolerances and guidelines formulated to avoid these effects are based on evidence derived from animal studies and experience with older children.

The major late effect of irradiation is growth disturbance (see Chapter 13 and Chapter 49). The possibility of bone and soft tissue deformity in children secondary to radiation therapy is recognized, but the normal growth pattern of any organ or structure in the young child can be severely disrupted by therapeutic doses of radiation. [40] Examples include mental retardation, aortic arch dysgenesis, and agenesis of the female breasts after irradiation of relevant structures. [41,42,43 and 44] The oncogenic effect of therapeutic irradiation has also emerged as a major problem. [42] There are no data to suggest that infants are particularly vulnerable to this effect.

It is generally believed that, because of ongoing development, radiation sensitivity of certain structures and organs in infants is increased. One such organ is the brain. The brain of the young infant is still immature. There is a very high mitotic activity in spongioblasts, and many of the major nerve tracts have not been myelinated, particularly in the frontal lobes. [43] Intellectual functioning is significantly lower in infants with brain tumors who received cranial irradiation as part of their therapy than in infants treated without irradiation. [44] Other major organ systems are also particularly vulnerable to damage, including the skeleton, kidney, liver, and lung. Skeletal growth has been shown to be more severely affected, dose for dose, with younger age. [45] Acute and chronic nephropathy can be caused by relatively low-dose radiation therapy given at any age, and the kidney of the newborn may be even more sensitive to irradiation because of its immaturity, as demonstrated by low glomerular filtration rates during the first 6 months of life. [46] Radiation damage to the liver can result in acute and chronic changes that may prove lethal. The liver's limited ability to conjugate bilirubin during the first week of life demonstrates immaturity, which suggests an increased radiation sensitivity. [47,48] Radiation therapy would also be expected to reduce the potential for normal development of the lung of the young infant and newborn. It can again be assumed that susceptibility to chronic pulmonary insufficiency increases with decreasing age at treatment. [49]

Because pediatric tumors are generally quite radiosensitive, relatively low total doses can be used. Protraction of therapy, using low-dose fractions and split-course techniques, coupled with alternating or simultaneous courses of chemotherapy, may be useful in preventing some of the late effects in infants. [39] The total dose, daily dose, and duration of treatment given to infants and newborns must be based on the natural history of the specific disease process. Chemotherapy given for systemic benefit probably provides an element of local tumor control as well, which may permit a significant reduction in total dose of radiotherapy required. [39] The volume irradiated should be kept as small as possible. Beam-shaping blocks, electron therapy, and other technical maneuvers should be used to avoid irradiating especially sensitive structures and to decrease the risk of long-term sequelae. “Shrinking fields” are also advantageous. This method minimizes the volume that receives the highest dose, avoiding the delivery of damaging doses of radiation to vulnerable structures. Surgical procedures can be performed after preliminary courses of chemotherapy or after administration of part of the total planned radiation dose. This provides time during which relatively normal growth and development can occur. Reexploration permits accurate localization of the tumor volume that must be irradiated. In some situations, a second-look operation allows direct implantation of radioactive materials into residual tumor, further minimizing the volume treated. [39]

Often, technical difficulties exist in ensuring immobility during treatment planning and delivery. Physical restraints, sedation, and even short-term general anesthesia may be required.

### *Chemotherapy*

The rationale for the use of cancer chemotherapeutic agents in the newborn and infant is no different from that for older children with disseminated solid tumors and acute leukemia. Because of the difficulties associated with surgery and radiotherapy in infants, however, chemotherapy also plays an important role in reducing the size of massive tumors that clinically appear local. Chemotherapeutic debulking may make these tumors more amenable to surgical or radiotherapeutic ablation and prevent systemic spread or suppress the growth of occult micrometastatic disease present at diagnosis. [50]

Several reports in the literature identify chemotherapy-related toxicities that are of special concern for infants. Infants are known to experience excessive vincristine-related neurotoxicity, manifested in extreme cases by hypotonia, poor cry, inability to feed, and fatal flaccid paralysis. [51] Increased myelosuppression in infants with Wilms' tumor given regimens containing vincristine, dactinomycin, and doxorubicin (Adriamycin) was seen in the second National Wilms' Tumor Study (NWTS-II). This resulted in the recommendation that dosages of all drugs be reduced by 50% in infants younger than 1 year of age. [52] This dosage reduction has not resulted in an increased rate of recurrence or metastasis. [52] These guidelines were also adopted by the Intergroup Rhabdomyosarcoma Study Committee for the management of solid tumors in infants and have been used in the intensive myeloablative regimens for the treatment of acute myelogenous leukemia (AML). [53] In a large series of infants with ALL, however, excessive chemotherapy-related toxicity, other than vincristine neurotoxicity, was not observed, and reduction of induction dosages for anticipated toxicity had an unfavorable impact on rates of remission induction and on remission duration. [3] A retrospective study of infants treated with a median of six cycles of 2 mg per kg cisplatin did show more acute toxicity, including electrolyte disturbances, but they did not have more long-term toxicity compared with older children.[54]

Reduction of initial dosages to prevent toxicity does not lessen the need for continued vigilance in monitoring possible toxic reactions. To maximize therapeutic efficacy, judicious incremental increases of subsequent doses should be considered if toxic effects have not developed. Chemotherapy-induced toxicity to specific organ systems (e.g., liver, lung, heart, and kidney) has rarely been reported in infants. In the few cases of hepatotoxicity, the use of multiple chemotherapeutic agents or of simultaneous radiation therapy to the target organ makes it difficult to implicate specific drugs. [52,53]

Data are lacking on the clinical pharmacology of most chemotherapeutic agents in newborns and infants. The optimal use of chemotherapy in this age group is best accomplished with pharmacokinetic monitoring. There are distinct technical difficulties in monitoring small infants, however, and the rapid changes in physiologic parameters that affect pharmacokinetics, particularly in the newborn, make such investigations difficult. Virtually every aspect of the distribution, excretion, and metabolism of anticancer drugs is quantitatively and qualitatively altered in newborns and young infants. Aspects unique to the pharmacology of the neonate include the rapid change in relative volume of fluid compartments that occurs after birth; different rates of hepatic metabolism; decreased efficiency of renal excretion; decreased protein-binding capacity; increased volume of cerebrospinal fluid, brain, and spinal cord tissue relative to body surface area (BSA); increased permeability of blood–brain barrier; and erratic gastrointestinal absorption. [55]

Rapid changes in the volumes of body water compartments occur during the first 9 months of life. In newborns, total body water constitutes almost 80% of body weight, and values similar to those in adults (50% to 55%) are seen in the older child. [56] Extracellular water volume is approximately 45% of body weight at birth but decreases to 20% in older children. Most of the drugs used in cancer chemotherapy are distributed in total body water or extracellular water. The convention of using BSA for drug dosing results in a standardization of the concentration of chemotherapeutic agent originally in the drug's volume of distribution, but it does not account for the changes in the distribution of body water compartments with age. [57] The BSA method therefore may not be appropriate for the very small infant (less than 6.0 kg), for whom calculations of dosage based on body weight may be more physiologic. [58]

The renal function of very young infants is less than would be predicted on the basis of body weight or surface area. Renal blood flow is lower in newborns, the ability of the renal tubules to concentrate or acidify the urine is restricted, glomerular filtration rate is low, and the organic ion transport system for active tubular secretion is underdeveloped, resulting in an increased reabsorption rate of weakly acidic drugs. [59,60] The maturation of various renal functions proceeds at different rates; therefore, chemotherapeutic agents that are not extensively metabolized and depend on renal excretion for elimination are cleared slowly in the newborn and young infant. This may result in prolonged plasma half-lives, with an increased risk of toxic reactions.

It has been demonstrated that neonates can metabolize drugs, but the ability of the immature liver to metabolize depends on the specific drug. [61] There are also wide interindividual variations in plasma clearance of specific drugs during the first few days of life, and a knowledge of the statistical mean rate of metabolism may be of little value to the clinician in choosing a drug dosage and regimen for a given infant. [55]

Because of the lower concentration of plasma proteins, the presence of a qualitatively different (fetal) albumin, high serum concentrations of competing substances such as bilirubin and free fatty acids, and lower blood pH, the binding of drugs by plasma proteins is lower in the neonate, resulting in a higher volume of distribution.[60,62]

Intrathecally administered methotrexate and cytarabine (Ara-C) are widely used for the treatment or prevention of meningeal leukemia. It has been demonstrated that age-related pharmacokinetics differences exist and that BSA does not accurately reflect the volume of the CNS. [63] The substantially greater ability of drugs to enter the CNS of the newborn compared with the adult has been thought to reflect incomplete myelination. Increased cerebrospinal fluid levels of methotrexate, despite normal renal clearance, have been demonstrated in infants receiving very-high-dose systemic infusions of methotrexate compared with levels in older children. [64]

The absorption of drugs from the gastrointestinal tract largely depends on pH-related diffusion and gastric emptying time. Low gastric pH and prolonged gastric emptying time in infants may result in relative inefficiency of orally administered chemotherapeutic agents. [65] Diminished bile acid metabolism due to hepatic immaturity may also result in prolonged clearance of chemotherapeutic agents normally excreted in bile and in unanticipated toxicity. [66]

Recommendations for dosage modifications in situations in which excessive myelosuppression should be avoided in newborns and young infants are provided in Table 15-5. These guidelines are based on the limited data available and must be applied within the context of the specific cancer being treated and the individual clinical situation. Unless otherwise stated, “decreased dose” implies a 50% reduction of the reference dose for older children when calculated on the basis of BSA. For infants weighing less than 6.0 kg, doses calculated on a mg per kg basis, using reference doses in milligrams derived for a $1m^2$ individual divided by 30 (assuming that a $1m^2$ individual weighs 30 kg), result in approximately the same 50% reduction. As many of these recommendations are empiric and not based on detailed pharmacology studies, escalation of subsequent doses should be considered if initial doses are well tolerated.

| Drug | Reason for modification | Dose modification* |
|---|---|---|
| 6-Mercaptopurine | Clearance dependent on levels of hepatic enzymes; decreased until 3 mo | Decrease by 50% until ≥3 mo |

ALL, acute lymphoblastic leukemia; AraC, methotrexate and cytarabine; DTIC, dacarbazine; GFR, glomerular filtration rates; VM-26, teniposide; VP-16/VM-26, etoposide.
*Many of these modifications have been derived empirically and are not based on detailed pharmacokinetic studies.
†According to Children's Cancer Group dosing criteria.

**TABLE 15-5. DOSAGE MODIFICATIONS OF CHEMOTHERAPEUTIC AGENTS IN INFANTS AND NEWBORNS**

The use of hematopoietic growth factors has reduced the myelosuppressive toxicity of most chemotherapeutic agents.[67] Growth factors should be considered in the treatment plan for infants undergoing myelosuppressive therapy to reduce toxicity and avoid further dose reductions.

***Pain***

The evaluation and management of pain in pediatric oncology patients present a unique challenge. This is especially true for infants who are nonverbal and have a limited ability to express physical discomfort.[68] Studies have documented that infants have physiologic stress responses to painful procedures and have improved outcomes when pain is treated.[69,70] Effective pain management in infants depends on a high level of awareness by health care providers. The sources of pain to consider in infants with cancer are their underlying disease process and invasive diagnostic or therapeutic procedures. Signs of pain in very young infants include cry, grimace, irritable behavior, withdrawal of affected body part, tachycardia, sweating palms or soles, elevated blood pressure, stress hyperglycemia, and decreased oxygen saturation. Older infants may developmentally be able to physically resist painful procedures and develop anticipatory fear.[69] The strategies for managing pain in infants include palliative or curative therapy to eliminate the source of pain, and pharmacologic analgesia. This is similar to the approach used for older children and is discussed further in Chapter 43.

***Supportive Care***

Management of the infant with cancer is best accomplished in a specialized pediatric tertiary care setting, wherein the unique medical, surgical, anesthesia, blood-banking, and nutritional requirements of seriously ill newborns and infants can be met.

Because venous access often becomes a problem very early in the management of infants, the elective placement of a tunneled indwelling right atrial catheter (i.e., Hickman and Broviac) should be considered to facilitate the administration of parenteral alimentation, blood products, and chemotherapy.[71] Cannulation of the external jugular vein rather than the usual cephalic vein is generally recommended for infants. Depending on the treatment plan, subcutaneous implantable devices are a possible alternative to tunneled indwelling central venous access. Specific guidelines to prevent and treat the infectious and thrombotic occlusive complications of these indwelling catheters are presented in Chapter 12 and Chapter 41.

Early empiric institution of nutritional support should be considered before the initiation of intensive therapies. Because there is growing suspicion that a malnourished state impairs host ability to tolerate anticancer therapy, possibly decreasing response to treatment, the early use of parenteral alimentation is warranted if less invasive approaches are precluded by gastrointestinal dysfunction.[72] This is necessary to prevent the added morbidity of multimodal therapy until it is safely established that normal weight gain and growth can be accomplished without supplementation.

Of great importance is the immediate availability of blood products with the lowest possible shelf life and methods to provide maximal transfusion support without risk of excessive volume overload and unnecessarily increased donor exposures (e.g., infant quadpacks and quintpacks, quadruple or quintuple blood collection systems, centrifugation of platelet-rich plasma, plateletpheresis, and directed donor programs).[73] All blood products administered to infants receiving intensive chemotherapy should be irradiated (at least 1,500 cGy) to prevent graft-versus-host disease.[74] Use of blood products that are negative for exposure to cytomegalovirus has also been recommended.

Potential long-term, organ-specific, treatment-related toxicities, anticipated to occur with increased frequency in infants with cancer, warrant the early institution of a coordinated, longitudinal evaluation of growth and specific organ (e.g., lung, skeletal, liver, and kidney) function to identify subclinical problems that may respond to early therapeutic intervention. Most important, longitudinal assessment of neuropsychologic development in infants at risk for neurotoxic sequelae may delineate early signs of learning disabilities that can benefit from remedial intervention.

**Specific Neoplasms in Infancy**

***Neuroblastoma***

Neuroblastoma is the most common cancer in infants (see Chapter 31).[1,2] It accounts for more than one-third of the malignancies observed in the first year of life and more than one-half of those in the neonatal period.[1,2,75] Between 21% and 35% of all neuroblastomas occur in infants younger than 1 year of age. The incidence of neuroblastoma in infants is probably underestimated because many tumors spontaneously regress before detection. Small neuroblastomas have been found incidentally during routine necropsies of young infants dying of other causes with a frequency 40 times greater than expected for clinically overt neuroblastoma.[76]

More than one-half of neuroblastomas in infants present in the abdomen, presumably originating in the adrenal gland. The extent of disease at diagnosis, as demonstrated by the most widely used staging system, is different for infants compared with older children. Most infants present with stage IVS (30%) or local disease (40%), and most older children (55%) present with distant metastases (i.e., stage IV disease).[5,77] Stage IVS patients are a unique group of infants who have small localized primary tumors that do not cross the midline and evidence of distant spread to liver, skin, bone marrow, or combinations of these sites, without radiographic evidence of cortical bone metastases.

The patient's age at diagnosis and stage of disease have a dramatic impact on treatment outcome in neuroblastoma. The overall disease-free survival for infants with neuroblastoma approximates 75%. Historically, survival rates for infants with stage IV disease have been dismal, but regimens using intensive multiagent therapy have greatly improved outcomes.[78,79]

Although age and stage have important prognostic implications, these two variables alone cannot predict outcome. Other important prognostic variables in infants include serum levels of neuron-specific enolase, serum ferritin, histopathology, cellular DNA content, and metastatic bone marrow disease detected by immunocytology.[80,81,82,83,84,85] and [86] Molecular genetic characteristics of neuroblastoma that are often found in infants and predict a favorable prognosis include tumor cell hyperdiploidy, absent karyotypic abnormalities (specifically chromosome 1p deletions), no amplification of the MYCN proto-oncogene, and absence of increased TRKA expression.[87,88,89] and [90]

The importance of these factors in predicting prognosis is demonstrated by the difference in survival for two groups of infants with stage III neuroblastoma. Infants with stage III disease, favorable histopathology, less than ten copies of N-*myc*, and normal ferritin have a 3-year progression-free survival rate in excess of 70%. In comparison, infants with stage III disease, unfavorable histopathology, increased ferritin, and more than ten copies of N-*myc* have a less than 30% 3-year rate of survival.[89] These prognostic factors have been evaluated prospectively in formulating treatment plans for infants with advanced-stage neuroblastoma.[78]

The management of infants with stage I or stage II neuroblastoma may be surgical resection only, depending on biologic characteristics. Some infants with stage II neuroblastoma have posterior mediastinal primaries not amenable to total surgical resection. Radiation therapy in doses of 900 to 1,800 cGy has been successfully

used as an alternative or adjuvant to surgical resection to treat intraspinal disease in these infants, but should be reserved for patients failing chemotherapy. [91,92] Stage III and IV are treated with a combination of surgery, radiation, and intensive multiagent chemotherapy. Infants younger than 1 year of age with stage IV neuroblastoma have a much improved outcome compared with children older than 1 year of age. Infants with non-MYCN–amplified tumors have a 93% 4-year event-free survival, whereas those with MYCN–amplified tumors have a dismal prognosis.

Few other clinical situations in pediatric oncology have been as controversial as the management of infants with stage IVS neuroblastoma. The finding of hyperdiploid cells by flow microfluorometric DNA analysis provides convincing evidence that this is a malignant lesion rather than a hyperplastic proliferation resulting from a one-mutation event in germinal cells, as previously proposed. [83,93] Among 165 stage IVS patients for whom adequate follow-up data are available, the survival rate is 77%.[94,95] Only 24% of the deaths in this group resulted from progression of disease to stage IV. The remainder were caused by respiratory compromise secondary to massive hepatomegaly, coagulopathy, or therapy-related complications. More than 50% of these patients received some form of chemotherapy, and some received radiotherapy. Of those who received no chemotherapy, 80% exhibited spontaneous regression of tumor. In the 7-year Children's Cancer Group (CCG) experience with 44 infants with stage IVS neuroblastoma, the only deaths were three patients younger than 2 months old at diagnosis, and the 2-year disease-free survival for infants 3 to 12 months of age was 97%. No significant influence of chemotherapy or radiotherapy on ultimate survival could be demonstrated. A nonrandomized study by the Pediatric Oncology Group (POG) demonstrated that chemotherapy consisting of cyclophosphamide and doxorubicin accelerated tumor regression but had no impact on overall survival.[96] In a subsequent nonrandomized POG study in which patients received either observation or immediate chemotherapy, the overall actuarial survival for all IVS patients was 90%.[97] In a small series of infants with stage IVS neuroblastoma, amplification of MYCN was strongly correlated with survival outcome. Although initial tumor regression was observed in some stage IVS patients with amplified N-*myc*, later progression occurred.[98]

Because chemotherapy does accelerate tumor regression, treatment with cyclophosphamide (5 mg per kg per day for 5 days) or the sequentially scheduled regimen of cyclophosphamide (150 mg per $m^2$ for 7 days) and doxorubicin (35 mg per $m^2$ on day 8) are warranted to initiate regression and to prevent life-threatening complications related to mass disease.[92,93] Radiotherapy in doses of 450 cGy (150-cGy daily fractions using lateral opposing fields) has also been effective. [92]

The biologic and genetic characteristics that have demonstrated prognostic significance in neuroblastoma must be taken into consideration when deciding on treatment options for stage IVS patients. A prospective correlation of clinical and biologic factors with natural history of stage IVS neuroblastoma is being undertaken by the CCG and POG to identify stage IVS infants who may require more intensive therapy. Until these data are available, it is recommended that infants younger than 1 year of age with clinical stage IVS disease, normal karyotype, nonamplified MYCN, and no complications due to the mass of their disease be closely followed without therapy. Infants with clinical stage IVS, abnormal karyotypes, amplified MYCN, or complications due to the mass of their disease should be aggressively treated. Ultimately, resolution of the controversy over how to treat clinical stage IVS neuroblastoma requires redefining of stage IVS to include genetic characteristics of the tumor.

There is considerable interest in the early detection of neuroblastoma in infants through mass screening programs using urinary catecholamine measurements at 6 months of age. Despite initial reports of a beneficial impact of such screening on survival, no population-based data from controlled studies exist to demonstrate reduced mortality.[99,100] The incidence of the disease in the first year of life has increased considerably in Japan, where a nationwide screening program has been in place since 1985.[101,102] However, the incidence of neuroblastoma among children older than 1 year of age has not changed, suggesting that a high proportion of prognostically favorable cases (many of which may have spontaneously regressed) are being detected. [103] Because mass screening has not been shown to reduce mortality, widespread implementation of this practice cannot be recommended.[104]

### *Brain Tumors*

Primary tumors of the CNS account for approximately 15% of cancer in infants (see Chapter 27).[2] There has been an increased incidence of CNS tumors observed in U.S. infants, and CNS tumors are now the second most common cancer in infants.[1,2] This unexplained increase in incidence is seen largely in male infants and therefore cannot be solely attributable to improved diagnostic techniques or reporting. [2]

The most common presenting feature of CNS tumors in infants is rapidly expanding head size and bulging fontanelle. Because of the expandability of the cranial vault, symptoms referable to increased intracranial pressure, other than vomiting, are rare in infants. Papilledema is rarely observed. Other clinical signs observed with greater frequency include paresis, seizures, cranial nerve palsies, lethargy, and nuchal rigidity. [105] In contrast to the experience in older children, an increased frequency of supratentorial rather than infratentorial tumors has been observed in infants, partially because of an increased relative frequency of cerebral hemispheric tumors.[106] Medulloblastoma and ependymal tumors account for approximately 50% of the histologic subtypes.

Overall, the prognosis for children with brain tumors is poor, and infants represent a subset of patients with particularly high morbidity and mortality. [107] The primary treatment of brain tumors in children is surgical resection and radiation therapy. Because of the doses of radiation used, serious sequelae are almost a certainty in infants with primary CNS tumors.[106] Several small studies have used various chemotherapy combinations in an attempt to defer radiotherapy in young infants until patients are at least 24 to 36 months of age.[108,109] These approaches have demonstrated efficacy without excessive toxicity in early follow-up reports. A CCG study of infants younger than 18 months of age with medulloblastoma or ependymoma treated with intensive chemotherapy and delayed or no radiation therapy extended survival for 20% of patients.[110] A similar POG study of brain tumor patients younger than 3 years of age treated with a cyclophosphamide/vincristine and cisplatin/etoposide combination of chemotherapy with delayed or deferred irradiation had an improved progression-free survival of 39% for infants younger than 24 months old.[111] Both studies demonstrated that deferred radiation therapy did not negatively impact progression-free survival. The POG study also showed that extent of surgical resection was an important prognostic factor. [111] Current treatment strategies for infants with brain tumors therefore include chemotherapy with delayed or deferred irradiation, intensification of dose and timing of initial chemotherapy, and incorporation of a second surgical resection as indicated. These strategies should continue to improve survival and long-term neuropsychologic outcome for infants with brain tumors.

### *Acute Lymphoblastic Leukemia*

Infants account for approximately 3% of all children with ALL, and they experience the worst prognosis of any group of children with this disease (see Chapter 19). In a series of early clinical trials of the CCG, fewer than 25% of patients survived without relapse at 3 years from diagnosis. Infants present with a constellation of clinical features associated with a poor prognosis, including hyperleukocytosis, hepatomegaly, splenomegaly, CNS leukemia at diagnosis, thrombocytopenia, and poor early response to treatment determined by day 14 bone marrow examination.[3]

The blasts of infants with ALL more frequently have a common ALL antigen (CALLA-CD10)-negative phenotype.[112] Infants with ALL also have increased frequencies of cytogenetic abnormalities: pseudodiploidy and hypodiploidy and translocations involving chromosome 11 with a break at band q23, associated with rearrangements of the MLL gene.[113,114 and 115] The specific 11q23 breakpoint abnormality, t(4;11), is the most frequent structural karyotypic abnormality in infants with ALL, detected in 30% to 50% of patients using standard cytogenetic techniques.[15,114] This specific translocation results in the synthesis of a protein that is a fusion product of the transcripts from the ALL-1 gene on chromosome 11 (also known as *HRX*, *MLL*, and *HTRX-1*) and the AF-4 gene on chromosome 4,[115,116] which plays a pivotal role in leukemogenesis. The prognosis is dismal in this subgroup.[116] Using molecular analysis, the MLL gene is uniformly rearranged in cases with t(4;11) and has been reported in 70% to 80% of infants with ALL.[116]

Particular focus on the molecular epidemiology of infant leukemia results from the frequent association of both ALL and AML with rearrangements of the MLL gene in up to 80% and 50% of infants, respectively. Identical abnormalities of the MLL gene at band 11q23 are involved in these rearrangements, [117] which may involve a variety of partner chromosomes, including 4, 9, and 19. Rearrangements of the MLL gene have also been observed in treatment-related AML, specifically cases associated with DNA topoisomerase II inhibitors, such as the epipodophyllotoxins, etoposide and teniposide. [118,119] The strong association between exposures to topoisomerase II inhibitors and the development of acute leukemia with MLL rearrangements has led to the intriguing hypothesis that maternal exposure to naturally occurring topoisomerase II inhibitors during pregnancy could increase the risk of infant leukemia. [120,121] A number of natural and synthetic topoisomerase II inhibitors exist, including flavonoids, catechins, caffeine, quinolones, thiram (an agricultural fungicide), certain benzene derivatives, and Chinese herbal medicines. [121] Preliminary data strongly suggest that maternal exposure to topoisomerase II inhibitors, particularly in the diet, is positively associated with AML in infants. [122,123]

Understanding the role of the MLL gene in the etiology of leukemia in infants has been significantly advanced by investigations of monozygotic infant twins. [124] Although the incidence of acute leukemia in infant twins is rare, detailed cytogenetic or molecular studies, or both, have demonstrated unique and identical clonal

molecular MLL gene rearrangements in cases of ALL with the t(4;11) and t(11;19).[125]

An unresolved question regarding the molecular etiology of infant leukemia, specifically ALL with the t(4;11), is whether expression of the MLL-AF4 fusion gene is sufficient to lead to the fully transformed phenotype.[126,127] The latency period to leukemia onset in the murine knockout mouse model and observations of human latency suggest that leukemogenesis requires genetic changes in addition to MLL translocations.

A recent finding of homozygous deletions of exons of the Ikaros gene with expression of dominant-negative Ikaros isoforms in six of seven infants who exhibited MLL-AF4 fusion suggests that disruption of normal Ikaros function may contribute to leukemogenesis associated with the t(4;11).[127] Inappropriate expression of non-DNA–binding Ikaros isoforms during early lymphopoiesis may dysregulate normal lymphocyte development, leading to maturational arrest at discrete stages of lymphocyte ontogeny, predisposing lymphocyte precursors to "second hits" and leukemic transformation.[128,129] Previous studies in mice have demonstrated that germline mutant mice expressing dominant-negative isoforms of Ikaros also develop ALL.[130] An abundance of dominant-negative mutant Ikaros isoforms that no longer bind DNA could interfere with centromeric recruitment and expression of specific genes during lymphocyte development. A lack of lineage-specific gene silencing could also explain the "lineage infidelity" demonstrated by myeloid antigen expression in ALL in infancy.[131,132] Obviously, further investigations of other molecular genetic abnormalities will be important in clarifying the leukemogenic role of such rearrangements and may provide clinically exploitable information for developmental therapeutics as well as disease prevention.

Despite major advances in cure rates for the general patient population, achieved through the identification of prognostic factors and the implementation of risk-adjusted therapy, the long-term event-free survival of infants with ALL approximates 40%.[133,134,135,136,137,138] and [139] This result, although only one-half that being achieved in standard-risk ALL patients, or in high-risk patients treated with intensified therapy regimens, represents an improvement over the historical experience. This modest success was accomplished through clinical trials and efforts to explore the biologic differences between leukemias in infants and older children. Retrospective reports of several series of infants with ALL, the largest dealing with infants treated on a number of consecutive clinical trials of the CCG, revealed 3-year, event-free survival rates of only 20%. Early treatment failure, characterized by both systemic and extramedullary relapse, rather than therapy-related toxicity, explained this poor outcome.[3]

The improvement in treatment outcome in infants with ALL, although decidedly less impressive than improvements seen in older children, have been accomplished through progressive intensification of systemic chemotherapy. Another significant accomplishment was the prevention of CNS relapse with the use of high-dose systemic, as well as intrathecal Ara-C, eliminating the need for cranial irradiation and resulting in a cumulative risk for CNS relapse of only 3%, despite a 14.2% prevalence of CNS leukemia at diagnosis. Intensification of therapy for infants has also included the combination of mitoxantrone and cytarabine,[136,138] intermediate-dose methotrexate infusions,[139] and combinations of cytarabine and etoposide.[135,140] Substantial reduction in the rate of isolated CNS relapse in two consecutive studies of the CCG have been observed in patients receiving CNS-directed therapy consisting of protracted (24-hour), very-high-dose (33.6 g per $m^2$) methotrexate infusions with leucovorin rescue plus intensive intrathecal therapy with Ara-C. Compared to historical controls, wherein the CNS relapse rates exceeded 20%, relapse rates of 9% and 3% in each of these two consecutive trials represented significant treatment advances.[134]

Developmental and neuropsychological evaluation of long-term survivors from these recent CCG trials have demonstrated mean scores on standardized cognitive and motor tests in the average range, with a normal distribution of scores by comparison with population-based standards.[141,142] These findings suggest a positive early developmental outcome for these children and represent a substantial improvement over the neurocognitive potential of previously treated infants.

To date, no other long-term clinical complications in patients, now followed for up to 8 years after successful completion of therapy, have emerged. Effective strategies to prevent CNS relapse while eliminating the potential for adverse neuro-cognitive and neuro-developmental sequelae are crucial to current future clinical trials investigating the optimal management of ALL in infants.

### *Acute Myelogenous Leukemia*

The outcome for infants with AML is not significantly different from that for older children (see Chapter 20).[53,143] However, an excess of myelomonocytic and monoblastic subtypes, which are associated with a less favorable prognosis, has been observed in infants. Infants with AML are more often found to have hyperleukocytosis, CNS leukemia at diagnosis, skin infiltration, and 11q23 abnormalities compared with older children.[53,143,144] The same therapeutic strategy is applied to infants and older children with AML. Despite the marked differences in these clinical and laboratory features, no differences in complete remission rate or survival have been observed between infants and children older than 2 years of age.

### *Retinoblastoma*

The incidence of retinoblastoma is 29 cases per million infants per year in the United States, representing approximately 13% of all cancers in infants (see Chapter 28).[2] Retinoblastoma is usually considered hereditary or nonhereditary (sporadic). Hereditary retinoblastoma represents approximately 40% of all cases, usually presents at a younger age (median, 13 months), and occurs with bilateral disease.[145,146] Only 10% of hereditary cases have a family history of retinoblastoma.[12] Susceptibility to retinoblastoma is inherited by deletions in the gene on chromosome 13q14, the retinoblastoma gene.[147] Sporadic cases are thought to be caused by acquired somatic mutations of this gene.

Infants with retinoblastoma most commonly present with leukocoria.[116] Strabismus, proptosis, blindness, an orbital mass, or other signs and symptoms of distal metastases can also be the initial finding.[116] Up to 70% of infants with retinoblastoma present with bilateral disease. Depending on the stage of disease, therapeutic options for infants with retinoblastoma include enucleation, irradiation, cryotherapy, and photocoagulation. Chemotherapy has limited effectiveness and is reserved for patients with metastatic disease.[148]

The prognosis for survival and salvation of vision for infants with retinoblastoma depends on the extent of disease at diagnosis. Overall, the survival rate for infants is high (85%).[145,146] In contrast, the mortality rate for retinoblastoma with distant metastasis or local extension at diagnosis is poor. Infants with hereditary retinoblastoma have a worse prognosis for long-term survival because of their increased risk of developing a second malignancy, most often osteosarcoma, later in life.[149]

### *Renal Tumors*

Renal tumors account for 11% of cancer in infants (see Chapter 30).[2] Although 16% of patients entered on the NWTS-II were infants, infantile congeners of Wilms' tumor are the more common renal neoplasms in newborns and infants (Table 15-6).[19] The most common is the congenital mesoblastic nephroma.[150] In the past, this growth was confused with Wilms' tumor, which may account for the more favorable reported prognosis of Wilms' tumor in infants. Mesoblastic nephroma (also known as *mesenchymal hamartoma of the kidney*) is not encapsulated and infiltrates into normal renal parenchyma. Complete surgical excision, with meticulous nephrectomy, is required. Local recurrences follow inadequate resection, and close follow-up is recommended. Only sparse reports of metastatic spread exist.[151]

| Renal neoplasm | Histologic and clinical characteristics |
|---|---|
| Congenital mesoblastic nephroma | Fibromyomatoid tumor; usually benign; often congenital; most common in infants <6 mo |
| Well-differentiated epithelial nephroblastoma | Closely packed, well-differentiated tubules; usually benign |
| Polycystic nephroblastoma or multilocular cystic nephroma | Macrocysts lined by flattened epithelium and fibrous septae; usually benign |
| Fetal rhabdomyomatous nephroblastoma | Predominantly in skeletal muscle; one-third bilateral; usually benign |
| Nodular renal blastema/ nephroblastomatosis complex | Nodular or confluent subcortical masses of hamartoid, hyperplastic epithelium; precursor of Wilms' tumor, particularly bilateral, hereditary type |

**TABLE 15-6. INFANTILE CONGENERS OF WILMS' TUMOR**

A potentially aggressive variant of mesoblastic nephroma is identified by foci of hemorrhage and necrosis, involvement of adjacent structures, and high cellularity and mitotic index. It is associated with invasion of adjacent structures or organs, multiple recurrences, and metastases. [152] This lesion probably represents an intermediate form between congenital mesoblastic nephroma and clear cell sarcoma of the kidney in the spectrum of infantile renal mesenchymal tumors originating from the premetanephric stromagenic stage of renal blastema. [153]

The clinical presentation of Wilms' tumor and its management in infants are not different from the experience of older children. However, toxic deaths were reported for 6% of infants with group I or II disease in NWTS-II, presumably related to excessive hematologic toxicity. [71] Modifications in therapy for infants, consisting of a 50% reduction in the dosage of all chemotherapeutic agents, were subsequently implemented and resulted in significant reduction in the severity and frequency of toxic effects and no deaths. Reduction in dosage did not jeopardize the therapeutic efficacy of adjuvant therapy. [52] Similar management has produced no excess toxicity or relapse in those infants in the NWTS-III study.

***Rhabdomyosarcoma***

Soft tissue sarcomas are relatively rare in infants, and this age group accounts for only 5% of patients in the Intergroup Rhabdomyosarcoma Studies. [154] No differences in sex ratio or clinical groupings (i.e., stage) were observed. The significant differences in clinical presentation, histopathology, and response are shown in Table 15-7. Unlike neuroblastoma and Wilms' tumor, rhabdomyosarcoma occurring before 1 year of age is not associated with a more favorable outcome. Before a study-wide amendment recommending a 50% reduction in chemotherapy doses for infants, excessive toxicity-related fatalities were observed.

| Characteristic | Children (n = 78) | Infants >1 y of age (n = 1,483) |
|---|---|---|
| Histology | | |
| Undifferentiated sarcoma | 18% | 7% ($p < .0005$) |
| Botryoid | 10% | 4% ($p < .005$) |
| Primary site | | |
| Bladder, prostate, or vagina | 26% | 10% ($p < .05$) |
| Clinical group III outcome | | |
| 3-yr survival | 41% | 62% ($p = .0002$) |
| Local recurrence rate | 29% | 11% ($p = .02$) |

**TABLE 15-7. INTERGROUP RHABDOMYOSARCOMA STUDIES I AND II: CLINICAL FEATURES AND OUTCOME OF INFANTS AND OLDER CHILDREN**

Review of the radiotherapy data for infants with embryonal rhabdomyosarcoma demonstrated an increased risk of local recurrence in those given total doses below 4,000 cGy in an attempt to reduce morbidity. In cases of orbital rhabdomyosarcoma, effective local control was achieved with doses between 3,000 and 4,000 cGy; reduction of radiation doses to less than 3,000 cGy to minimize acute and long-term side effects in infants with soft tissue sarcomas may jeopardize local tumor control. [155] The use of high-dose-rate remote brachytherapy as an alternative to external beam radiation therapy was reported to be safe, well tolerated, and effective in the treatment of a small group of very young children with rhabdomyosarcoma. [156] The efficacy of this treatment option awaits verification.

**Conclusion**

Improved survival for infants with cancer can be achieved with intensive therapy and the awareness of treatment-related toxicity. The most important challenges that remain include continued investigation of the unique etiologic and biologic characteristics of certain tumor types in infants; the pharmacology of chemotherapeutic agents in the infant population, particularly in newborns; and the design of specific therapeutic strategies that provide adequate or increased disease control while lessening potential acute and long-term toxic effects. Meeting these challenges will improve the outcome of infants with cancer.

## CANCER IN ADOLESCENTS AND YOUNG ADULTS

### Epidemiology

Data from the NCI's SEER program indicate that the overall rate of cancer in 15- to 29-year-olds was twice that in younger persons. Among adolescents 15 to 19 years of age, the rate in the United States during the 1990s was 203 new cases per year per million persons in this age group. [157] This rate is approximately 50% higher than the incidence of cancer in children younger than 15 years of age. The incidence of cancer in 15- to 19-year-olds is similar to the incidence of cancer among 0- to 4-year-olds in the United States, is twice that found in 5- to 9-year-olds, and is nearly twice that observed in 10- to 14-year-olds.

This rate is the highest reported since the SEER program began in 1973, with the incidence in 15- to 19-year-olds observed to increase an average of 0.9% per year from 1973 to 1997. Most of the increase occurred during 1973 to 1984 (estimated annual increase of 1.6% per year). During the more recent interval, 1985 to 1997, the rate is estimated to be only 0.1% per year. This pattern of a slowing in the increasing incidence is similar to the change in incidence observed in younger age groups.

The most common cancers among 15- to 19-year-olds in the United States are Hodgkin's disease (16%), germ cell tumors (15%), CNS tumors (10%), non-Hodgkin's lymphoma (NHL) (8%), thyroid cancer (7%), malignant melanoma (7%), and ALL (6%) (Table 15-8). As emphasized by Smith and co-workers,[158] this distribution is strikingly different from the pattern in younger and older persons (see Chapter 1). Many of the common malignancies in children younger than 5 years of age are virtually absent in 15- to 19-year-olds, including the embryonal malignancies of Wilms' tumor, neuroblastoma, medulloblastoma, ependymoma, hepatoblastoma, and retinoblastoma. Similarly, those cancers that predominate in adults, such as carcinomas of the breast and aerodigestive and genitourinary tracts, are distinctly unusual among adolescents. Although rare, many of the cancers of younger and older patients have nonetheless been reported to occur in adolescents (see Chapter 38). [159] [160] [161] and [162]

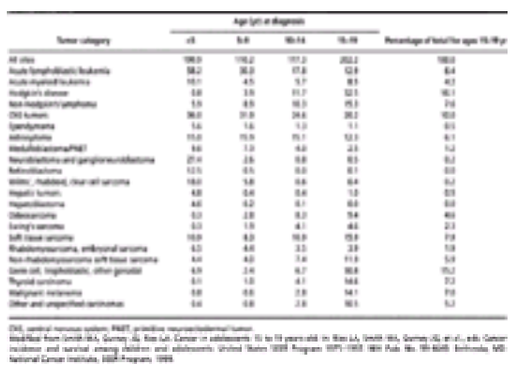

**TABLE 15-8. AGE-SPECIFIC CANCER INCIDENCE RATES PER MILLION AND PERCENTAGE OF TOTAL CASES BY CANCER TYPE AND AGE GROUP, ALL RACES, BOTH SEXES, SURVEILLANCE EPIDEMIOLOGY AND END RESULTS, 1986–1995**

At least six of the common malignancies in 15- to 19-year-olds increased in incidence between 1973 and 1995 ( Table 15-9). NHL and testicular carcinoma underwent the greatest increases over this interval, each averaging more than 2% per year for 24 years. ALL, osteosarcoma, and other germ cell and gonadal tumors showed similar increments (Table 15-9). This pattern is in contradistinction to the increased incidence of cancer in children in whom the increase was restricted to the two most common cancers, ALL and brain tumors.

| Tumor type (ICCC category) | 1975–1979 | 1980–1984 | 1985–1989 | 1990–1995 |
|---|---|---|---|---|
| All sites | 183.0 | 187.7 | 199.3 | 203.8 |
| Acute lymphoblastic leukemia | 10.6 | 13.2 | 12.4 | 13.0 |
| Non-Hodgkin's lymphoma | 10.7 | 14.5 | 14.4 | 16.3 |
| Osteosarcoma | 6.6 | 8.9 | 9.7 | 9.3 |
| Testicular germ cell tumor | 22.1 | 26.7 | 24.9 | 28.4 |
| Ovarian germ cell tumor | 7.9 | 8.3 | 11.8 | 13.3 |
| Gonadal carcinoma | 2.7 | 2.4 | 4.3 | 5.3 |

ICCC, International Classification of Childhood Cancer.
Modified from Smith MA, Gurney JG, Ries LA. Cancer in adolescents 15 to 19 years old. In: Ries LA, Smith MA, Gurney JG, et al., eds. Cancer incidence and survival among children and adolescents: United States SEER Program 1975–1997. NIH Pub. No. 99-4649. Bethesda, MD: National Cancer Institute, SEER Program, 1999).

**TABLE 15-9. AVERAGE ANNUAL AGE-SPECIFIC INCIDENCE RATES PER MILLION ADOLESCENTS 15 TO 19 YEARS OLD FOR SELECTED TUMORS, ALL RACES, BOTH SEXES, SURVEILLANCE EPIDEMIOLOGY AND END RESULTS, 1975–1995**

Osteosarcoma, Ewing's sarcoma, gonadal germ cell tumors, and Hodgkin's cancers peak in incidence during the adolescent and young adult age interval. The two sarcomas peak during the 15- to 19-year interval, and the latter two cancers peak during the 20- to 29-year age range ( Fig. 15-1). The type of soft tissue sarcoma that occurs in 15- to 19-year-olds is also distinct from that of younger patients. Rhabdomyosarcoma predominates among the sarcomas of childhood, accounting for more that 60% of the soft tissue sarcomas in children younger than 5 years of age. In 15- to 19-year-olds, rhabdomyosarcoma accounts for only 25% of the soft tissue sarcomas. Non-rhabdomyosarcoma soft tissue sarcomas represent 75% of the soft tissue sarcomas. These include synovial sarcoma, liposarcoma, malignant fibrous histiocytoma, and malignant peripheral nerve sheath tumors.

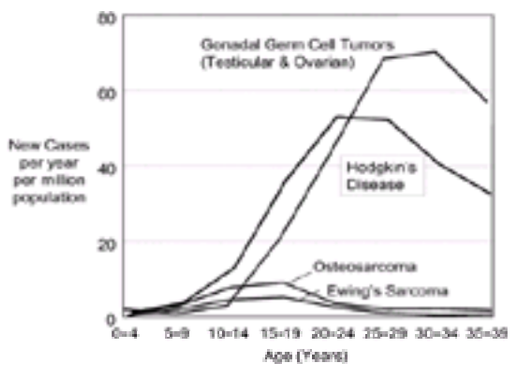


**FIGURE 15-1.** Incidence of four cancers that peak in the adolescent to young adult age range. Data from Surveillance Epidemiology and End Results, 1986–1995. From Ashikari H, Jun MY, Farrow JH, et al. Breast carcinoma in children and adolescents. Clin Bull 1977;7:55–62, with permission.

Leukemias and lymphomas are also distributed differently in older adolescents than in young children. ALL declines steadily with age from the 0- to 5-year age bracket upward, such that by age 15 to 19, ALL accounts for only 6% of the cancers in contrast to the 30% level in children younger than 15 years of age. In 15- to 29-year-olds, NHL is more common than ALL. NHL increases steadily with age (Fig. 15-2), but the subtype distribution changes from a predominance of lymphoblastic and Burkitt's lymphomas during early childhood to a predominance of diffuse large cell lymphoma during adolescence and early adulthood. AML is nearly as common as ALL in 15- to 19-year-olds and more common than ALL in 20- to 29-year-olds. Chronic myelogenous leukemia increases steadily with age from birth on, but is it not as common as either ALL or AML during the 15- to 29-year age range. Juvenile myelomonocytic leukemia is uncommon at all of the four 5-year age groups before age 20, but especially during the 15- to 19-year interval.

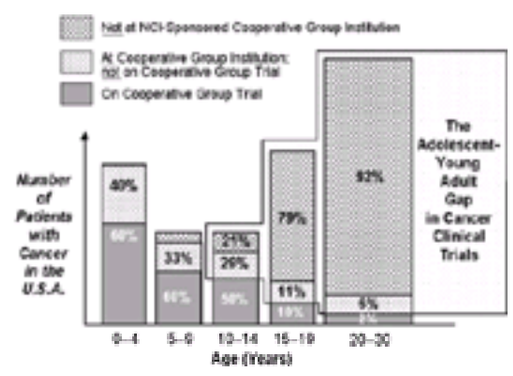


**FIGURE 15-2.** Adolescent and young adult cancer patients on clinical trials. NCI, National Cancer Institute.

Ethnic and racial differences in incidence are particularly apparent among African-American and non-Hispanic white adolescents and young adults. Among 15- to 19-year-olds in the United States, the overall incidence of cancer is 50% higher among whites than blacks. Among specific cancers, melanoma and Ewing's sarcoma are strikingly higher in whites, as is the case during any age range. ALL, germ cell tumors, and thyroid cancer are also more common in whites than in blacks, each by at least twofold. Only soft tissue sarcomas, considered as a group, are more common in blacks than in whites among the common cancers in this age group.

Incidence by gender is also different, with an overall incidence equal among males and females 15 to 19 years of age in contrast to a 20% higher rate in boys younger than 15 years of age. Individual tumor types have unequal sex distributions in the older adolescent populations, however. The most striking difference is in thyroid carcinoma, with females being ten times more likely to get this disease. The next greatest gender difference is ALL, in which males are more than twice as to likely develop this disease. Females are also 50% more likely to be diagnosed with melanoma, and approximately 15% more likely to sustain Hodgkin's disease. [159] Males are nearly twice as likely to have NHL or Ewing's sarcoma, 50% more likely to develop osteosarcoma, and 20% to 30% more likely to have brain tumors or NHL. [159]

**Etiology**

As in younger patients, little is known about the causes of cancer in adolescents and young adults. Very few cancers in adolescents and young adults have been attributed to environmental or inherited factors. Most cases of clear cell adenocarcinoma of the vagina or cervix in adolescent females were found to be caused by diethylstilbestrol (DES) taken prenatally by their mothers in an attempt to prevent spontaneous abortion. [163] Radiation-induced cancer may occur in adolescents and young adults, in most cases when the radiation exposure occurred during early childhood. Many of the adolescent and young adult cancers that have been linked to

etiologic factors are second malignant neoplasms in patients who were treated with chemotherapy or radiotherapy, or both, for a prior cancer. Skin cancer, lymphoma, sarcoma, and hepatic cancers occur at higher frequency in persons with inherited conditions such as neurofibromatosis, ataxia telangiectasia, Li-Fraumeni syndrome, xeroderma pigmentosa, Fanconi pancytopenia, hereditary dysplastic nevus syndrome, nevoid basal cell carcinoma syndrome, multiple endocrine neoplasia syndromes, and Turner syndrome. In the aggregate, however, these cancers account for a small proportion of the cancers that occur during adolescence and early adulthood. The vast majority is unexplained, similar to the state of knowledge of cancer during childhood.

Given that the duration of exposure to potential environmental carcinogens is directly proportional to age, older adolescents and young adults should be more likely to develop tobacco-, sunlight-, or diet-related cancers than younger persons. Nonetheless, there has been little evidence that the cancers in 15- to 29-year-olds are substantively related to these known environmental carcinogens, which of course is not the case for older adults. It appears to take considerably longer than one or two decades in most persons for these environmentally related cancers to become manifest.

## Diagnosis

### *Symptoms and Signs*

With few exceptions, the signs and symptoms of cancer in adolescents are similar to signs and symptoms of the same cancer in younger or older patients. Because of psychological and social factors, patients in this age range may be at higher risk for a delay in diagnosis. Adolescents may present with advanced disease—we have had older adolescents with extraordinarily large masses that they harbored for months—because they were too embarrassed to bring the problem to attention.

### *Pathologic Considerations*

Diagnosis is facilitated by an increased ability of the older patient to describe and localize the symptoms and signs caused by the malignancy, and the greater ease with which biopsies can be obtained.

## Management

### *Surgery*

In general, surgery is more readily performed in the larger patient, and anesthesia is easier to administer. That young adults are generally healthier than older patients is an advantage. The main disadvantage young adults have relative to children is that the fully grown patient generally has fewer compensatory mechanisms to overcome deficits and disabilities rendered by surgical resection of large tumors.

### *Radiation Therapy*

Adolescents and young adults are spared the vulnerabilities of developing tissues to the adverse effects of ionizing radiation. This is particularly true for the CNS, the cardiovascular system, connective tissue, and the musculoskeletal system, each of which may be irradiated to higher doses or larger volumes, or both, with less long-term morbidity in the older patient.

### *Chemotherapy*

For the same chemotherapeutic agents, acute and chronic toxicities are generally similar in children, adolescents, and young adults. Exceptions for the older patients in this age range may be a greater degree of anticipatory vomiting, a somewhat less rapid recovery from myeloablative agents, and fewer stem cells in the peripheral blood available for autologous rescue. Adherence to therapy regimens, particularly oral chemotherapy, is much more problematic in teenagers than in younger or older patients.[164,165 and 166]

### *Psychosocial and Supportive Care*

The greatest difference in the management of adolescents and young adult patients is in supportive care, particularly psychosocial care. These patients have special needs that are not only unique to their age group but also broader in scope and more intense that at any other time in life. The challenges include autonomy and independence, peer pressure, education, graduation, social development, sexual maturation, intimacy, marriage, reproduction, fertility, employment, parenting, and insurability.

When cancer threatens during this critical period of life, the patient, family, friends, and care providers are faced with unique challenges. High school students, college students, recent graduates, newlyweds, new employees, and new parents are expected to take on more challenges than at any other time in life. How can they plan and begin their future when they suddenly realize they may not have one? What will happen if they cannot graduate, keep their friends, finish their education, attain a good job, get married, have children, or be whatever they aspired to be? How can they possibly learn to be independent when becoming a patient makes them dependent on many others, the effectiveness of medical therapy, and the help of their families to survive at all, let alone survive the usual obstacles of adolescence and early adulthood?

These questions and many others emphasize the special needs of patients in this age range. Some of the adverse effects of therapy can be devastating to an adolescent's self-image, which is often tenuous under the best of circumstances.[167] Mutilating surgery to the face and extremities, weight gain, alopecia, acne, and stunted growth are examples. Although there is a dearth of publications that address these issues,[168] there are several that provide substantive advice on how to manage these issues.[169,170,171,172,173,174,175,176,177,178,179,180 and 181]

### *Lack of Participation in Clinical Trials*

Generally, 90% to 95% of children younger than 15 years of age with cancer are managed at institutions that are members of an NCI-sponsored clinical trials organization. In contrast, approximately 20% of 15- to 19-year-olds with cancer are seen at one of these institutions, and only approximately 10% of the patients are entered into a clinical trial (Fig. 15-2).[182,183] Among 20- to 29-year-olds, the participation rate is even lower, with fewer than 10% seen at member institutions of the cooperative groups, either pediatric or adult. Approximately 2% of 20- to 25-year-olds are entered into clinical trials of the pediatric or adult cooperative groups. This gap has been observed throughout the United States and spares no geographic region or ethnic group.[183] This dramatic reduction in clinical trials participation in older adolescents may help explain a lower than expected level of progress in older adolescents and young adults (see following section). There is evidence that older adolescents who participate in clinical trials have a more favorable outcome than those who do not.[182,183,184 and 185]

### *Survival*

Table 15-10 shows 5-year relative survival rates overall and for different cancer types for 15- to 19-year-olds. For all patients in the 15- to 19-year-old age group, the 5-year survival rate for the recent reporting period was 77%, which was higher than that for the other 5-year age groups younger than 20 years of age. Comparison of a recent reporting period (1985 to 1994) with that of an earlier time period (1975 to 1984) indicates that substantial survival gains have been made in most cancers of this age group during the past quarter century (Table 15-10) (see Chapter 1). The only cancer to show no gain, thyroid carcinoma, is also the one that has, by far, the highest 5-year survival rate, 99%.

| Tumor category | 1975–1984 (%) | 1985–1994 (%) |
|---|---|---|
| Total | 69 | 77 |
| Acute lymphoblastic leukemia | 35 | 51 |
| Acute myeloid leukemia | 22 | 42 |
| Hodgkin's disease | 88 | 90 |
| Non-Hodgkin's lymphoma | 56 | 69 |
| Astrocytoma | 62 | 75 |
| Medulloblastoma | 63 | 75 |
| Osteosarcoma | 49 | 59 |
| Ewing's sarcoma | 36 | 56 |
| Soft tissue sarcoma | 70 | 63 |
| Rhabdomyosarcoma | 40 | 45 |
| Germ cell tumors | 79 | 90 |
| Thyroid carcinoma | 99 | 99 |
| Melanoma | 84 | 92 |

Modified from Smith MA, Gurney JG, Ries LA. Cancer in adolescents 15 to 19 years old. In: Ries LA, Smith MA, Gurney JG, et al., eds. Cancer incidence and survival among children and adolescents: United States SEER Program 1975–1997. NIH Pub. No. 99-4649. Bethesda, MD: National Cancer Institute, SEER Program, 1999.

**TABLE 15-10. FIVE-YEAR RELATIVE SURVIVAL RATES BY CANCER TYPE AND TIME PERIOD, AGE 15–19, ALL RACES, BOTH SEXES, SURVEILLANCE EPIDEMIOLOGY AND END RESULTS, 1975–1984 AND 1985–1994**

However, improvement in survival in older adolescents has lagged behind the improvement in younger patients. In 1975, the 5-year survival for all patients with cancer was estimated to be 64% for patients 15 to 19 years of age when diagnosed with cancer and 55% for those younger than 15 years of age when diagnosed. In 1990, the 5-year survival increased to 76% for 15- to 19-year-olds and to 75% for younger patients. Thus, the relative improvement in survival was considerably greater in the younger patients than in older adolescents, as illustrated in Figure 15-3 for each 5-year age interval below age 20. By projecting this trend, the 5-year survival should have reached 80% for 15- to 19-year-olds and 85% for younger patients by 2000. If so, a reversal in the survival order will have occurred, from a 10% advantage in 5-year survival for older adolescents diagnosed in 1975 to a 5% disadvantage today.

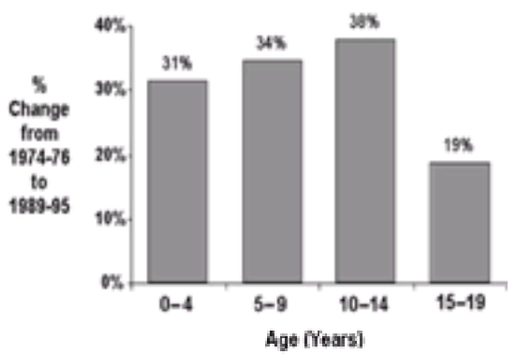


**FIGURE 15-3.** Percent change in 5-year survival rates from 1974 to 1976 and 1989 to 1995, by age. (From Ries LA, Eisner MP, Kosary CL, et al., eds. SEER Cancer Statistics Review, 1973–1997. Bethesda, MD: National Cancer Institute, 2000, with permission.)

The worst outcomes among the common cancers in 15- to 19-year-olds are in AML, ALL, and the sarcomas, particularly rhabdomyosarcoma, Ewing's sarcoma, and osteosarcoma. Each of these has a considerably lower mean 5-year survival rate than the corresponding malignancy in younger patients. With the exceptions of thyroid carcinoma, melanoma, and germ cell tumors, most of the remaining common cancer types in older adolescents have a worse prognosis than the same cancer in younger patients.

The mortality burden is a function of the survival and the incidence rates. More than 80% of the U.S. national cancer mortality burden in 15- to 19-year-olds is due to four malignancy groups: sarcomas, leukemia/lymphomas, CNS tumors, and germ cell tumors. The leukemias are the primary contributor to the cancer mortality burden for cancers developing in 15- to 19-year-olds. Although thyroid carcinoma and melanoma are among the more common cancers in this age group, they contribute little to the overall cancer mortality burden.

## Conclusions

Surviving adolescence and young adulthood is difficult enough, even when all is well and health is not limiting. Adding cancer to this phase of life is extraordinarily more challenging and demanding. There is evidence that progress in diminishing the cancer problem for these patients has lagged behind accomplishments in younger patients. The relative gap in clinical trial participation by older adolescents and young adults with cancer is at least a partial explanation for the relative lack of progress.

Despite a need for adolescent oncology that was recognized years ago, [186] a specific discipline for this special target population is just beginning to evolve. This will help bring the problem in focus and begin to address solutions. Meanwhile, resources should be devoted to educating the public, professionals, insurers, and legislators about the special needs of these patients. [187] The U.S. NCI and the NCI-sponsored pediatric and adult cooperative groups have launched a national initiative to improve the accrual of adolescents and young adults with cancer in clinical trials.

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# STEM CELL TRANSPLANTATION IN PEDIATRIC ONCOLOGY

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## INTRODUCTION

Reconstitution of hematopoiesis via infusion (transplantation) of hematopoietic progenitor or stem cells (SCs) is an established treatment approach for many malignant and nonmalignant diseases that affect the hematopoietic or immune system, or both. In addition, transplantation is also used to support myelosuppression secondary to the dose-limiting toxicity of chemoradiotherapy regardless of the tissue origin of the tumor cell.

The first recorded use of bone marrow (BM) infusion to treat human disease described an empiric decision to infuse blood and BM from a sibling into a patient with marrow failure.[1] The theoretical basis for hematopoietic stem cell transplantation (HSCT) evolved later from observations that mice could withstand an otherwise lethal exposure to whole-body irradiation if the spleen was protected, and similar radiation protection could be provided by an infusion of BM. [2,3 and 4] The first attempts at human BM transplantation (BMT), reported in 1957, were largely unsuccessful but did demonstrate that large amounts of anticoagulated and screened BM could be infused intravenously without ill effects. [5] In 1959, two patients with acute lymphoblastic leukemia (ALL) who received supralethal total-body irradiation (TBI) and infusion of BM from identical twins had successful hematologic recovery, although their leukemia recurred. [6] By the end of the 1960s, several different groups had achieved success in transplanting patients with a variety of immunodeficiency disorders. [7,8,9,10 and 11] Over the next decade, an increasing degree of success was attained using transplantation of BM from matched sibling donors for patients with hematologic malignancies. [7,8,9,10,11,12 and 13]

Both in this early period and subsequently, animal models and clinical trials have jointly contributed to our understanding of transplantation biology. Among the key observations that have contributed to making HSCT a more commonly available treatment modality have been an improved understanding of the critical role of histocompatibility in allogeneic HSCT; the pathophysiology and prophylaxis of graft-versus-host disease (GVHD); the identification, collection, and quantitation of hematopoietic progenitors in BM, peripheral blood (PB), and/or umbilical cord blood (UCB) that are capable of sustaining long-lived human hematopoietic reconstitution; and the identification of essential elements of supportive care during the period of most profound hematopoietic ablation and greatest immune compromise. Indeed, advances in establishing and maintaining long-term vascular access, infectious prophylaxis, treatment of opportunistic infections, transfusion support, nutritional management, and treatment, prophylaxis, and improved management of toxicities due to chemotherapy or TBI preparative regimes, or both, are also improving outcome in HSCT.

By current treatment standards, HSCT should be considered for patients for whom this treatment is physiologically rational and for whom alternative therapeutic options are likely to result in inferior long-term disease-free survival (DFS). The likely benefit of HSCT must outweigh the risks both in terms of acute and chronic complications and, particularly in the case of nonmalignant or more indolent diseases, the potential benefits of other standard or experimental treatments. Potential candidates must have a suitable source of hematopoietic stem cells (HSCs) available at an appropriate time in the course of the disease.

This chapter reviews the current status of HSCT in pediatric oncology and addresses its use in patients with ALL, acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), myelodysplasia (MDS) or myeloproliferative syndromes, non-Hodgkin's and Hodgkin's lymphoma, and neuroblastoma and other solid tumors. In addition, an overview of HSCT procedures, including conditioning regimens and selection of donor and HSC source, as well as a discussion of common early and late-onset posttransplant complications are provided.

## OVERVIEW

### Hematopoietic Stem Cell Sources

Potential sources of HSCs have increased in recent years and are likely to expand even further. Thus, the pediatric oncologist must consider all the available SCs and their relative merits for each potential patient and disease state.

#### *Autologous Stem Cells*

Autologous HSCs can be harvested from the BM, and more recently the PB, of pediatric patients. With the advent of elective UCB banking, UCB may also become an important source. The most significant limitations to use of autologous HSCs have been the potential for contamination with tumor cells and the risk of post-HSCT MDS. MDS that occurs after autologous HSCT may reflect reinfusion of damaged HSCs and/or failure of the HSCT to eradicate damaged residual HSCs in the BM because allogeneic effects against remaining HSCs are absent. [14,15,16 and 17] It is prudent to screen all patients for cytogenetic abnormalities before proceeding to HSC collection.[18]

Few randomized reports compare the long-term results of autologous BMT versus PBSCT, although there are numerous reports suggesting that the cellular composition, immune potential, and degree of tumor contamination may differ. [14,19,20,21,22 and 23] Studies have demonstrated that PBSCT often results in faster engraftment than BMT, although no long-term benefit has been demonstrated.[24,25] Little or no data directly comparing the use of chemotherapy or cytokine-mobilized BM to the use of similarly stimulated PBSCs is currently available, particularly in pediatrics. The $CD34^+$ content of the HSC product is related to the rapidity of engraftment.[20,26]

#### *Allogeneic Stem Cells*

Conventionally, allogeneic donors have been HLA (histocompatibility locus A)-identical siblings, but improved overall results of HSCT coupled with novel immunosuppressive or graft engineering strategies, or both, have encouraged the expansion of the donor pool to include mismatched family members and HLA-matched and partially matched unrelated donors. In the allogeneic setting, therefore, the decision to perform a transplant involves an additional step of weighing the benefits to the patient against the risks to the healthy donor. In general, BM and PBSC donation are considered safe procedures, although some complications have been reported.[27,28 and 29] In the United States, children are routinely used as donors with parental consent. [30] The legality of giving such consent is not straightforward because laws and their interpretations differ by state and country. Additional ethical and legal issues must be considered when using BM or UCB collected from unrelated donors.[27,31,32,33 and 34]

Volunteer unrelated donors include only healthy persons between 18 and 60 years of age who fulfill health requirements similar to those applied to blood donors. All potential allogeneic donors undergo an extensive medical evaluation; in the case of UCB donation the mother serves as a “surrogate” and the evaluation is adjusted appropriately. The examiner should obtain the same history used for blood product donation. Physical examination and screening laboratory tests with complete blood cell count, biochemistry profile, hepatitis screen, and other testing for transmissible infectious agents, including cytomegalovirus and human immunodeficiency virus, should be completed. Many donor candidates will have preexisting medical problems that require further evaluation. BM donors are usually admitted to the hospital the morning of the harvest. The aspiration procedure is conducted in an operating room under sterile conditions and with appropriate anesthesia. Most BM is harvested only from the posterior iliac crests, but when the recipient is significantly larger than the donor or when large cell volumes are needed, BM may also be harvested from the anterior iliac crests. The total volume of BM usually collected amounts to 10 to 20 mL per kg of recipient weight. These volumes represent realistic target volumes that usually yield sufficient HSCs for engraftment. Evidence suggests that BM from children, especially infants, has a higher concentration of nucleated cells and probably a higher proportion of marrow-repopulating cells than BM from older donors. [35] Accordingly, BM volume may be adjusted downward. Donors of appropriate age may store autologous blood before BM donation to be returned during the procedure; this is generally not indicated unless calculations indicate that the loss in red cell mass presents a significant risk. Most BM donors can be discharged the same day after recovery from anesthesia. Cytokine-mobilized PBSC can be harvested from either related or unrelated adult donors as well as related pediatric donors; this has been successfully accomplished with the placement of antecubital access in both arms, although access issues may present a more significant obstacle with younger and smaller donors. [27,36,37] In general, donors receive recombinant human granulocyte colony-stimulating factor, 10 to 16 µg per kg per day, for 3 to 7 days to mobilize sufficient PBSC for collection. As both the short- and long-term toxicities of this treatment for normals are not well established, the ethical issues around using unrelated adult and related pediatric PBSC donors are considerable.

Since the first such report in 1990, UCB has been collected, cryopreserved, and used as a HSC source. [38,39,40 and 41] UCB is most often collected at the local hospital by trained obstetric personnel and then transferred to a public or private bank for processing and storage. UCB units are tested for infectious agents in accordance with still evolving standards developed by governmental and specialty oversight organizations. The extent of histocompatibility and genetic testing required is still being established.

**Histocompatibility**

Suitability of allogeneic donors is first determined by defining their degree of histocompatibility with the recipient. Currently, matching is generally confined to the major class I and II loci, which constitute the human major histocompatibility complex (MHC). The MHC consists of closely linked genetic loci inherited as a genetic unit or haplotype.[42] These genes are mapped within the HLA region located on the short arm of chromosome 6. However, it is becoming increasingly appreciated that there are minor histocompatibility antigens that likely also play important roles in the outcome of allogenic HSCT. [43,44 and 45] T lymphocytes from one person recognize allelic differences in non-self MHC antigens, resulting in an immune or alloreactive response. MHC antigens can be divided into two groups. The class I molecules HLA-A, HLA-B, and HLA-C are generally regarded as endogenous peptide-presenting cell surface molecules. The class I region also contains additional structurally related class I genes, of which HLA-E, HLA-F, and HLA-G are potentially functional. Class I molecules are composed of an a chain and b $_2$ microglobulin, are highly polymorphic, and are expressed on most nucleated somatic cells and on platelets. The class II molecules, HLA-DR, HLA-DQ, and HLA-DP, are involved in exogenous antigen processing and peptide transport. The HLA class II molecules are heterodimers consisting of two HLA-encoded polypeptide chains a and b.

HLA terminology is designated by the World Health Organization Nomenclature Committee for Factors of the HLA system and is updated at regular intervals. [46] The nomenclature distinguishes the technique used to determine the HLA type and its level of resolution. The broadest designation is based on serologic typing whereas the highest resolution is based on actual DNA sequence. For example, “HLA DR 6” designates a specific class II serotype (i.e., determined by reactivity to serologic reagents); antigens (“HLA DR 6”) recognized by these reagents can be subdivided into DR 13 and 14 by additional serologic reagents or by low-resolution molecular techniques. In turn, DR 13 can be further classified by intermediate or high-resolution molecular analysis of the b chain itself. This is incorporated into the nomenclature, resulting in a report of the allele as DRB1 1301 through 1336 (i.e., the number determined by the polymorphisms described to date). The type of assay used and its sensitivity and specificity are important considerations in determining potential histocompatibility, particularly for mismatched family or for any unrelated donor HSCT.

The genetic unit of HLA class I and II regions on one chromosome is referred to as an *HLA haplotype*, and the two HLA haplotypes in one person are called the *HLA genotype*. Class I and class II antigens are codominant and are transmitted as dominantly inherited mendelian traits. Each child expresses one set of paternal and one set of maternal HLA antigens corresponding to the HLA genes inherited as one paternal and one maternal HLA haplotype. The probability that a child will inherit any one of the four possible HLA genotypes is .25. When, by chance, an individual inherits a phenotypically identical HLA allele from each parent, the result is a person homozygous for that locus; a person may be homozygous for one allele at a single HLA locus or for the entire haplotype. The HLA genes can be separated by genetic recombination. Recombination or crossover between class I genes is rare. Genetic recombination between HLA class I and II regions also occurs infrequently. Thus, although a careful examination of family haplotypes is essential to determining donor suitability, the HLA complex can generally be considered as a single genetic unit that is most often inherited as a block. Some haplotypes exist in strong linkage disequilibrium, which are particularly likely to persist without mutation. For example, the haplotype HLA-A1, HLA-B8, HLA-DRB1 1701 is particularly frequent in the northern European white population and has become one of the most common haplotypes in African-Americans, Hispanics, and Native Americans.

**Conditioning Regimens and General Procedure**

The preparative or “conditioning” regimen has traditionally been seen as having three major roles: providing “empty” hematopoietic space for the infused HSCs, providing sufficient host immunosuppression (in the case of allogeneic HSCT) such that donor cells are not rejected and, last, eliminating residual tumor burden. The relative roles of these three goals have shifted somewhat over time and differ greatly by disease process and type of HSCT. It has become increasingly clear that immunologic events (“allogeneic effects”) play key roles in recipient hematopoietic ablation and subsequent disease elimination. In consequence, the potential of allogeneic T cells to facilitate engraftment by eradication of host hematopoiesis, and potentially by other mechanisms, is being incorporated into current approaches to conditioning. Conversely, the association of T-cell depletion (TCD) as a method of GVHD prophylaxis with subsequent graft failure or with an increased degree of residual host hematopoiesis after HSCT, or both, often referred to as *mixed chimerism*, has spurred the addition of further agents to conventional HSCT conditioning regimens.[47,48,49,50,51,52,53 and 54] More recently, these observations have also provided the rationale to explore donor lymphocyte infusions (DLIs) with or without HSC support as a primary part of the conditioning regimen in so-called nonmyeloablative HSCT. [55,56,57,58,59 and 60] In addition, there is increased recognition of the role of allogeneic T cells in eradicating recipient tumor burden as exemplified both by the decreased relapse rate of patients with GVHD and the efficacy of DLI in patients relapsing after HSCT for CML.[61,62,63,64,65 and 66] The mechanisms by which these immune effects can be harnessed more directly in the preparation of the patient for HSCT and their management thereafter are important topics of current research likely to significantly impact HSCT in the next decade. [59,60]

Nonetheless, the current mainstay of conditioning for HSCT remains combination myeloablative therapy, most commonly built on a base of ablative doses of either TBI or busulfan (BU). Despite the observation that donor engraftment was only transient and relapse common, the first allogeneic HSCT were carried out using TBI and established this preparative approach. [6] The use of single-agent cyclophosphamide (CY) produced similar results [67] whereas combination therapy (CY/TBI) was rapidly shown to be more effective. As HSCT neared its first decade, BU was developed as an additional ablative agent that did not require the construction of costly radiation facilities. [68] More varied and somewhat more cytotoxic regimens with or without TBI were piloted in patients undergoing autologous HSCT. In this setting, freedom from post-HSCT GVHD prophylaxis (i.e., methotrexate) as well as the absence of GVHD-mediated cytotoxicity permitted somewhat more intensive treatment.[69] The specific regimens currently in use for patients with various disorders vary significantly and are reviewed by disease entity in the following sections where pertinent.

Allogeneic BM or PBSCs are most often infused intravenously directly into the recipient. Autologous BM, PB, or UCB from any donor is collected before the preparative regimen, cryopreserved until the regimen has been completed, and then infused immediately after thawing. Several techniques have been developed for

special *ex vivo* treatment of SCs before infusion or cryopreservation. These include red cell depletion in the setting of major ABO incompatibility, TCD of allogeneic donor cells to decrease the risk of GVHD, and so-called purging of autologous HSCs to remove contaminating malignant cells. Approaches used for purging vary widely and include positive selection of HSCs or depletion of selected (tumor) cells by various techniques. The duration of neutropenia after HSC infusion varies widely depending on the regimen and SC source. During this period, supportive care is critical. Patients are at risk for bacterial, fungal, viral, and protozoal infections.[70,71 and 72] Infectious morbidity is most commonly associated with allogeneic HSCT; however, the move toward increased intensity of both prior treatment and conditioning for patients undergoing autologous HSCT appears to be increasing opportunistic infections in this population.[73] Accordingly, infectious prophylaxis as well as aggressive management of fever in these neutropenic, immunosuppressed hosts is extremely important.[72] Patients require attentive management of transfusions with attention to adequate irradiation to prevent engraftment of GVHD from cellular constituents and, in the allogeneic setting, to any ABO differences.[74,75,76 and 77] Nutritional support is extremely important, although the best mechanism and intensity are as yet not established.[78,79,80,81 and 82] Prophylaxis of regimen-related toxicities is in its infancy but likely to evolve over the next decade.[83,84,85 and 86]

## TRANSPLANTATION FOR HEMATOLOGIC MALIGNANCY

From its inception, HSCT has been an important treatment option for patients with relapsed leukemia and a matched family donor. The improved identification of patients likely to fail primary therapy as well as expansion of the potential donor pool has made HSCT an option available to additional children. Thus, the role of HSCT in children with hematologic malignancies must continually be reconsidered and re-evaluated, particularly as improvements in patient management have measurably improved the outcome of HSCT since the time that most patients currently being reported were actually treated.[87]

### Hematopoietic Stem Cell Transplantation for Acute Lymphoblastic Leukemia

Historically, the relatively good outcomes of patients with ALL treated with standard chemotherapy limited the contribution of HSCT to the treatment of recurrent disease. For patients newly diagnosed with ALL, HSCT has had more limited application. However, it has become clear that certain patient subsets of ALL do not respond as well to these same approaches and that HSCT may be useful as primary therapy for such patients ( Table 16-1). A retrospective study of children with Philadelphia chromosome-positive ($Ph^+$) ALL treated with chemotherapy found a DFS of only 25%.[88] BMT from HLA-identical related donors significantly improved DFS (65%) and overall survival (72%). The advantage of HSCT became more apparent over time because, in contrast to transplant recipients, the risk for relapse persisted for chemotherapy-treated patients beyond the second year of remission. The benefit of HSCT was not seen for patients transplanted from alternative donors largely due to treatment-related deaths. Reflecting the superior results often reported by a single center or limited centers, a study of HSCT for $Ph^+$ ALL conducted at two institutions reported 65% DFS and 12% relapse rate. Data from the International Bone Marrow Transplant Registry (IBMTR), which combined children and adults with $Ph^+$ ALL, showed 38% DFS and 34% relapse rate for patients in first complete remission (CR1).[89] Because of its poor prognosis, the use of alternative donors has been actively pursued in $Ph^+$ ALL, with DFS of 37% and 68%[90] reported in two studies using TCD. However, children with advanced disease and those who received SCs from less than a full match by current HLA allele–specific typing had reduced DFS due in part to increased regimen-related toxicity. Clearly, the use of alternative donors can be curative and is an appropriate option for newly diagnosed children and adolescents with $Ph^+$ ALL.

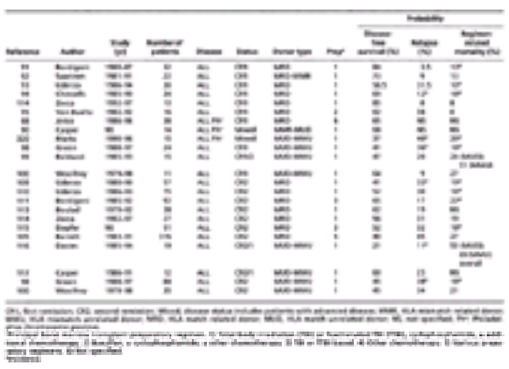

**TABLE 16-1. HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)**

Except for $Ph^+$ ALL, no consensus exists regarding the indications for HSCT for children with ALL in CR1. High initial white cell count, infant ALL, 11q23 rearrangement, T-cell lineage, poor response to prednisone, induction failure, and an unfavorable diagnostic risk index have been considered indications to perform HSCT. With such indications, DFS between 58% and 85% and relapse rates from 3.5% to 31.5% have been reported.[91,92,93,94 and 95] Thus, children whose curability with intensive chemotherapy is less than 50% with contemporary front-line chemotherapy may benefit from early allogeneic HSCT.[96] It is interesting that for children, in contrast to adults, the outcome after alternative donor HSCT has compared favorably to the outcome of HSCT from HLA identical related donors in some studies.[96,97] DFS reported from single institution studies range from 41% to 64% and relapse rates from 9% to 29%.[97,98,99 and 100] Although it is unknown whether improved supportive care will alter this statistic, deaths in remission remain a major obstacle and are responsible for a significant portion of the mortality after HSCT. The major causes of death varied between reports and were related to the specific institutional treatments. Until the optimal approach is determined, HSCT from alternative donors for children with ALL CR1 will remain controversial.

Originally, HSCT primarily addressed the plight of children with ALL no longer considered curable by conventional therapies. Salvage therapy after relapse or induction failure remains the principal indication for HSCT for children. Some contemporary intensive chemotherapy regimens are also able to provide prolonged disease control for selected children with recurrent ALL, but extended follow-up will be needed to assess the durability of these chemotherapy responses.[101,102] Chemotherapy for relapse has been most effective for children with a long CR1 duration and for extramedullary relapse. BM relapse, especially during the first 12 to 18 months of treatment,[103,104 and 105] or early central nervous system (CNS) or testicular relapse all have poor outcome when treated with chemotherapy alone.[101,106] The relative improvement in outcome if HSCT is used in this setting is unclear, and any comparison of chemotherapy and HSCT must address potential biases such as patient selection and time delay between relapse and HSCT.

In studies reported to date of HSCT from matched related donors (MRDs), children with ALL second complete remission (CR2) have reported DFS rates between 32% and 65%.[105,107,108,109,110,111,112,113,114 and 115] Studies that compare DFS between HSCT and chemotherapy find an advantage to HSCT, although the differences have not always been significant; alternatively, the advantage has been limited to certain patients such as those with early BM relapse.[105,109,113,115] Data from two large cooperative groups report superior DFS (or event-free survival) regardless of the duration of CR1, but in one report this difference did not become significant until 4 years post-relapse.[103,105] Among children with early BM relapse, HSCT has consistently provided superior DFS.[101,103,104 and 105,109,113] It is less clear whether HSCT improved the DFS for children whose relapse occurred off therapy.[105,113,115] The probability of relapse has been uniformly lower after HSCT (13% to 45%) compared to chemotherapy, but this advantage has been partially mitigated by the higher numbers of deaths in remission (10% to 20%). The outcome for children in CR2 undergoing alternative donor HSCT has been very similar to that of matched sibling HSCT.[97,103] Although in one study DFS was only 21% for children with ALL in CR1 and CR2 most studies reported DFS greater than 40% (DFS: 42% to 60%).[98,99,116,117 and 118] Because the precision with which degree of match between donor and recipient can be ascertained has improved and as other elements of donor selection become more refined, the results of alternative donor HSCT are likely to ever more closely approximate the matched family setting.[98,119,120]

Children in remission beyond CR2 may also benefit from HSCT. Allogeneic HSCT from MRDs and unrelated donors have resulted in extended DFS ranging between 10% and 42%.[98,99,111,116,117 and 118,121] In contrast, the salvage rate for children with ALL in relapse at the time of transplant remains dismal at less than 10%, and a transplant should probably not be performed except in the context of a clinical investigation.

Even with the expanding number of potential unrelated donors, not every child needing a transplant will have a donor. Furthermore, the time required to establish a suitable donor may be prohibitive under certain circumstances. Some studies have explored the use of mismatched or haploidentical family donors, but no substantial disease-specific data have been established. More disease-specific data are available for the results of cord blood HSCT. Data from the Eurocord cooperative group found 39% DFS after related (the majority HLA identical) and 30% DFS after UCB (the majority HLA mismatched) transplantation for acute leukemia (no difference

between ALL and AML).[122,123] Patients in CR1/2 fared better, with a DFS of 49%. If allogeneic HSCT is neither a practical nor a desired option, autologous transplantation provides another option. For CR1 patients, DFS approached 50% in some studies, but more aggressive and effective chemotherapy treatment has matched these results. As salvage therapy for children whose initial BM remission exceeded 24 months, autologous HSCT has resulted in DFS between 53% and 60%.[124,125] Likewise, for children with isolated extramedullary relapse, autologous HSCT has provided effective disease control. [126] However, a review of the Medical Research Council United Kingdom ALL data found no significant difference in outcome between children receiving either autologous HSCT or chemotherapy after relapse.[103] Similar findings have been reported by other centers.[109,127]

The practical advantage of autologous HSCT has been the low regimen-related mortality (RRM), 10% or less, whereas the most obvious disadvantage has been an unacceptably high relapse rate of up to 72%.[93] Contamination of autologous BM by residual leukemic cells has been recognized as a source leading to relapse in AML[128] although this issue has not been directly explored in ALL. Purging harvested BM with monoclonal antibodies or chemotherapeutic agents has been incorporated into a number of trials, but at present there is no absolute evidence that any of these manipulations provide an advantage to children with ALL undergoing autologous HSCT.[124,129,130] Any future efforts to develop autologous HSCT will likely focus on methods to purify HSC (positive selection) rather than removal of leukemic cells (negative selection) by purging techniques. However, both techniques could be used for additive effect.

Regardless of the source of HSC or the disease status of the patient at the time of HSCT, the majority of posttransplantation failures are due to disease relapse. There are no studies to suggest a consistent difference in outcome by preparative regimen. The alternate strategy of inducing an antileukemia immune esponse post-HSCT has been explored through the use of DLI administered to relapsed patients. Although DLI has not been as effective for patients with ALL when compared to patients with CML, it has demonstrated some activity.[131,132] However, DLI may be followed by severe acute GVHD or pancytopenia, or both. More effective and less toxic measures are needed. Measurements of residual leukemia pretransplant have been correlated with relapse posttransplantation. [133,134] In some studies, persistent recipient chimerism present within selected hematopoietic compartments and assayed by polymerase chain reaction (PCR)–based quantification of HLA alleles has been correlated with relapse.[135] Using assays such as these, it may be possible to define patients for whom adjuvant therapy is appropriate. Those children may be candidates for novel treatments, including posttransplant immune modulation, such as administration of cytokines, antitumor vaccines, and/or leukemia-specific cytotoxic cells.

## Hematopoietic Stem Cell Transplantation for Acute Myeloid Leukemia

### *Allogeneic Hematopoietic Stem Cell Transplantation*

Substantial improvements in the treatment of AML have altered the indications for HSCT ( Table 16-2). Cooperative groups report up to 60% DFS for children with favorable AML subtypes as defined by epidemiologic or cytogenetic characteristics. [136,137] Cytogenetic findings are the most important indicator of AML prognosis. The AML subtypes most consistently reported to be associated with good outcome after chemotherapy include FAB M2 with translocation t(8;21), FAB M3 with translocation t(15;17), and FAB M4 with inv(16), and for these patients, HSCT may not be necessary as primary therapy. In contrast, poor response to chemotherapy and poor DFS has accompanied cytogenetic findings of monosomy 5 and monosomy 7. Some 30% to 40% of pediatric AML cannot be classified as favorable or unfavorable according to cytogenetic findings. The role and timing of HSCT for these latter patients remains a subject of ongoing investigation. Transplantation from MRD produced superior DFS for adults with AML and favorable cytogenetics compared to patients treated with autologous HSCT or chemotherapy, but the data are less clear as to whether transplantation improves the DFS for patients with unfavorable cytogenetics. [138,139] In addition, other poor risk groups, such as infants younger than 2 years of age, have been particularly difficult to treat with standard chemotherapies. The role of HSCT in this population remains unclear as well. In a trial limited to such infants, 38% survived without relapse.[140] Future clinical trials will need to define treatment options and therapeutic outcome for poor-prognosis patients. At present, HSCT studies for childhood AML have not addressed this issue prospectively.

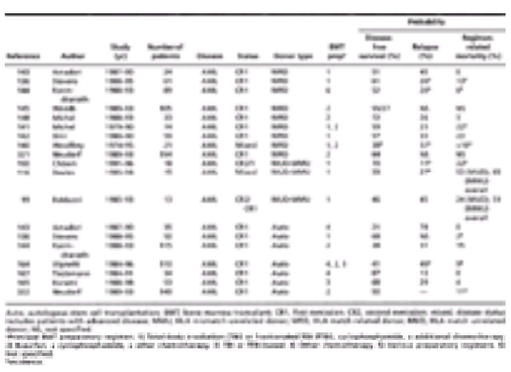

**TABLE 16-2. HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA (AML)**

Single-arm studies of MRD transplantation for children with AML in CR1 reported DFS similar to studies in which patients were assigned to either HSCT or chemotherapy based on donor availability. [141,142] These latter comparative studies, conducted by several cooperative groups, attempted to determine whether allogeneic transplantation from MRDs improves the DFS over that achieved after chemotherapy or autologous HSCT for patients with AML in CR1. In those studies that report DFS, allogeneic HSCT was statistically superior (DFS range, 51% to 52%) to the alternative therapies (DFS: autologous, 21% to 38%; chemotherapy, 27% to 36%).[143,144,145,146] and [147] Using survival as the measure of outcome, one study found no difference between the three options (survival: allogeneic, 69%; autologous, 73%; chemotherapy, 66%).[136] In each trial, children were allocated to allogeneic transplantation if a suitable MRD was available; the remaining patients were randomized to chemotherapy or autologous transplantation. Most of these studies analyzed patients on the basis of intention to treat; patients who refused randomization were excluded from the comparisons. All patients with an available donor were analyzed as part of the allogeneic transplant cohort regardless of whether the patients actually underwent transplantation; likewise for patients randomized to autologous transplantation or chemotherapy. The timing of transplantation varied between two, three, or four courses of chemotherapy administered before transplantation. Thus, comparisons between these studies are difficult, particularly with a view toward understanding the impact of HSCT on cure of AML. One other study compared MRD transplantation and chemotherapy and found statistically superior DFS for the HSCT recipients: 72% and 48%, respectively. [148] This study, like the others, found that allogeneic transplantation patients experienced fewer relapses (probability of relapse: 26% MRD transplantation; 47% chemotherapy). The decline in RRM noted in that study, and subsequently observed by others, will be an important part of reassessing the future role of allogeneic transplantation. [87,143,149]

Experience is limited using alternative donor transplantation for children with AML in CR1. Nevertheless, reported DFS of 33% to 70% appears comparable to that achieved with MRD.[99,116,150] The higher RRM that accompanied the use of unrelated or mismatched donors in the past, 65% in one study, has decreased markedly; similarly, the improved outlook for selected patients treated with chemotherapy in some studies has limited the use of this strategy. [116,151]

Approximately 40% of children relapse after contemporary treatment for AML, and with further treatment one-half of these attain a second remission. [152,153] HSCT remains the most important and successful modality for patients beyond CR1. For HSCT from MRDs performed from 1991 to 1997, the IBMTR data found that 50% of atients who were in CR2 and younger than 20 years old survived 3 years after allogeneic transplantation. If all age groups were included, the survival was 35% for autologous transplantation and 40% for allogeneic transplantation. [154] Other registry data show similar DFS of approximately 40% after allogeneic or autologous transplantation.[155,156] Several single-institution studies report DFS after alternative donor transplantation equivalent to MRD transplantation. [97,150] Factors that determine which relapsed patients are the best candidates for DFS after HSCT include length of CR1, achievement of CR2, and, perhaps, absence of extramedullary disease.[152,153,156,157] Survival for patients transplanted beyond CR2 has been poor in most studies.

Alterations to the conditioning regimen, particularly via the use of antibody therapy targeted to myeloid or stem/progenitor cells, or both, may impact significantly on DFS.[158] Preliminary studies have been encouraging. Adjuvant therapy posttransplantation may also be a useful adjunct in preventing relapse and improving treatment outcome. Quantifiable PCR-based assays have demonstrated persistence of leukemia fusion genes in BM and PB of some patients in clinical remission after HSCT. In some, but not all, instances, this observation has preceded overt relapse. [159] Withdrawal of immunosuppression or administration of DLI has led to clearing of the

leukemic fusion gene.[159,160 and 161] More clinical experience will delineate the role of pre- and post-HSCT modalities in patient management.

### *Autologous Hematopoietic Stem Cell Transplantation*

For children with AML who relapse after conventional chemotherapy, and have no suitably matched allogeneic donor, autologous HSCT is an option if cryopreserved autologous marrow obtained during remission is available. In some reports, the DFS after autologous transplantation in CR2 has been comparable to that achieved for patients receiving autologous transplantation during CR1 or allogeneic transplantation in CR2. [155,162,163] Outcome data from prospective cooperative group trials have been conflicting as to the advantage of autologous HSCT over chemotherapy. In two separate trials conducted by the Associazione Italiana di Ematologia ed Oncologia Pediatrica (AIEOP) and the Pediatric Oncology Group (POG), DFS was equivalent between groups of children randomized to chemotherapy or autologous HSCT (AIEOP DFS: autologous, 21%; chemotherapy, 27%) (POG event-free survival: autologous, 38%; chemotherapy, 36%). [143,144] The Medical Research Council trial found that DFS was superior for children undergoing autologous HSCT (68%) compared to children receiving chemotherapy (46%); however, overall survival was equivalent.[136] Finally, data from the Children's Cancer Group noted an advantage to chemotherapy over autologous HSCT. [146] These studies differ in important ways, and comparison has been problematic. Retrospective data from pediatric registries find that DFS rates ranged from 41% to 68%. Single-institution trials have reported DFS up to 87%.[155,164,165,166 and 167] As with allogeneic transplantation, patients with favorable cytogenetics have improved outcome. [168]

Leukemic contamination of harvested marrow has been demonstrated to contribute to relapse after autologous HSCT. [128] From its inception, various methods have been used to purge BM of residual leukemia cells, generally by treating the BM with chemotherapy or antibodies against myeloid cells. [162] No method has yet been proven to improve DFS, and some purging methods have delayed engraftment and thereby increased transplant morbidity.

## MYELODYSPLASTIC SYNDROME

In addition to the adult subtypes of MDS, children may also present with juvenile myelomonocytic leukemia (JMML) and a variety of other and often unique myeloproliferative syndromes. Aggressive chemotherapy rarely results in prolonged remission, and chemotherapy before HSCT has not been shown to improve DFS.[169,170 and 171] Currently, allogeneic SCT remains the only curative modality. DFS rates between 36% and 87% have been reported for children with primary MDS undergoing allogeneic transplantation from MRDs or alternative donors ( Table 16-3). [169,171,172,173,174,175 and 176] Because of higher relapse rates, patients with JMML and RAEB-T generally fared worse than patients with the other MDS types.[172,173 and 174] As might be expected, patients with MRDs had improved DFS compared to patients transplanted from alternative donors, although, as in CML, the difference in time to donor ascertainment may contribute to this difference in outcome.

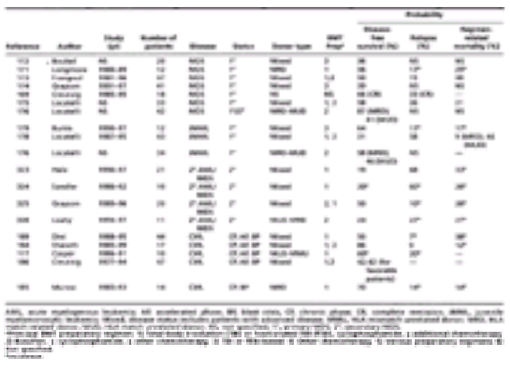

**TABLE 16-3. HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR MYELODYSPLASTIC SYNDROME (MDS), AND SECONDARY MDS AND CHRONIC MYELOGENOUS LEUKEMIA (CML)**

JMML is a myeloproliferative disorder unique to children and is characterized by hepatosplenomegaly, monocytosis, thrombocytopenia, and elevated hemoglobin F. The clinical course of a small proportion of patients with JMML may be indolent, with extended survival reported. For most children, however, progression has usually been inevitable, and allogeneic transplantation has been the only curative option. Aggressive chemotherapy has been effective in some reports but ineffective in others; 13-*cis*-retinoic acid has induced clinical remission for some patients. The reported outcome after transplantation for JMML has been poor, with overall DFS less than 40%.[177,178] Inability to eradicate the disease, resulting in relapse, has been the principal problem. More recent trials have reported improved DFS up to 68%, particularly when BU is used in the preparatory regimen. [176,179]

## CHRONIC MYELOGENOUS LEUKEMIA

As $Ph^+$ CML is uncommon in children, much of current practice is derived from adult clinical trials. Virtually all patients are in chronic phase at presentation. This chronic phase has a variable and an unpredictable duration, but without allogeneic HSCT, $Ph^+$ CML is ultimately fatal. Although interventions such as administration of a-interferon appear to prolong the stable phase and thus prolong survival, they are not curative. The novel tyrosine kinase inhibitor STI 571 has significant hematologic activity in stable phase CML, although its ultimate impact and risk benefit profile in pediatric patients is completely unknown. [180]

In adult trials, delay in transplant beyond a year from diagnosis has been associated with poorer DFS, and thus it has been recommended that transplantation not be unduly delayed.[181] The outcome is also inferior if HSCT is undertaken after the first chronic phase. More recently, it has been noted that treatment with a-interferon before HSCT may influence outcome for some patients. For patients needing transplantation from alternative donors, a-interferon administered for 6 months was associated with increased incidence of severe GVHD.[182] Data from the IBMTR indicated an increased risk of graft failure after transplantation from MRDs but a lower risk of relapse and ultimately no difference in DFS.[183] These observations must be confirmed, but for now caution is warranted regarding a-interferon administration to children before allogeneic HSCT.

In a single-institution study, more than 70% of adults younger than 50 years old who were transplanted within the first year after diagnosis are alive 5 years posttransplantation. There is less experience in children, but results appear comparable to those achieved in adults ( Table 16-3). Children with $Ph^+$ CML transplanted early in the disease have DFS rates between 70% and 86%.[184,185] Predictably, with longer time to transplant or inclusion of children beyond the first chronic phase, DFS rates decline to 42% to 62%.[117,186] The outcome was similar between MRDs and MUDs,[187] although registry data have reported lower survival rates for MRDs versus MUDs (67% versus 50%).[154,186,188,189] The early experience in “mini-transplant” for CML is very encouraging and likely to be successfully applicable to children. The resulting decrease in regimen-related toxicity should further improve the already impressive outcome for allogeneic transplant and will need to be carefully considered when triaging new therapeutic approaches.

## SOLID TUMORS

HSCT has been used for a variety of solid tumors occurring in children. However, in many cases the largest reported experience is contained within mixed adult and pediatric series, making the pediatric experience somewhat difficult to assess. Table 16-4 summarizes representative trials of HSCT for solid tumors.

| Center/reference | Number of patients | Disease and status | Disease-free survival (%) |
|---|---|---|---|
| MSK[191] | 23 | Medulloblastoma/recurrent | 34 |
| Duke[192] | 48 | Primary brain/poor prognosis or recurrent | 15 |
| MSK[193] | 28 | Primary brain/recurrent | 50 |
| MSK[197] | 16 | Pontine glioma/resistant | 0 |
| UCSF[194] | 379 | Neuroblastoma/post-induction without progressive disease | 43 |
| DFCI[195] | 39 | Neuroblastoma/post-induction without progressive disease | 58 |
| EBMT[196] | 63 | Ewing's/first or second remission | 29 |
| European collective[a] | 52 | Rhabdomyosarcoma/post-induction without progressive disease | 30 |
| EBMT[198] | 25 | Wilms'/poor prognosis/recurrent | 50 |

DFCI, Dana-Farber Cancer Institute; Duke, Duke Comprehensive Cancer Center, Duke University Medical Center; EBMT, European Group for Blood and Marrow Transplantation; MSK, Memorial Sloan-Kettering Cancer Center; UCSF, UCSF Cancer Center and Cancer Research Institute, University of California, San Francisco.
[a]European Bone Marrow Transplant Solid Tumor Registry.

**TABLE 16-4. OUTCOME OF AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH SOLID TUMORS**

### Brain Tumors

CNS tumors comprise the second largest group of pediatric cancers. The prognosis is dismal for those children failing surgery, radiation therapy, and/or conventional chemotherapy. Attempts were limited by myelosuppression, particularly in those patients who had received prior spinal irradiation leading to the use of autologous SC rescue after high-dose chemotherapy. Most preparative regimens have used thiotepa and etoposide with or without carboplatin, as these agents are known to cross the blood–brain barrier and are dose-limited by myelosuppression. The results for patients with primitive neuroectodermal tumor or medulloblastoma and high-grade gliomas outside of the brainstem are variable, although durable DFS appears achievable for some patients. [190] Outcome is closely correlated with tumor burden at the time of HSCT, ranging from less than 10% in children with bulky disease to 50% for those with minimal or no disease. [191,192,193] and [194] HSCT has also been used with encouraging preliminary results in infants and young children to avoid or reduce neuroaxis radiation. [194,195] However, good outcome depended on the ability of surgery or chemotherapy, or both, to produce a state of minimal disease before HSCT. [196] Patients with brainstem gliomas show no improvement in either survival or DFS with HSCT, presumably related to the refractory nature of these tumors to pre-HSCT conventional dose chemotherapy. [197]

### Neuroblastoma

Conventional treatment of children with high-risk neuroblastoma has generally resulted in a DFS of less than 20%. Efforts to improve outcome have focused on intensifying induction therapy in addition to using high-dose therapy with autologous SC support for consolidation. Newly diagnosed high-risk patients treated with regimens culminating in HSCT have been reported to have a 3-year DFS of 43% after conditioning with myeloablative chemotherapy and TBI. [198] Purged BM was used as the SC source. Post-HSCT therapy with oral *cis*-retinoic acid, which decreases proliferation and induces differentiation in neuroblastoma cells, [199] further improved survival in this cohort. However, relapse remained a significant problem. Further intensification of treatment, using PBSC to hasten engraftment, has since been explored in sequential transplants using non–cross-resistant agents and $CD34^+$ selected PBSC for rescue. It appears that closely spaced transplants (4 to 6 weeks apart) using both chemotherapy and TBI may increase DFS without unacceptable incremental toxicity. [200] A randomized study is planned to assess the advantage of single versus double HSCT as well as the role of oral differentiating agents. Although HSCT has thus been effective in newly diagnosed, chemoresponsive patients, those with relapsed or refractory disease have continued to fare poorly. Recent approaches to such patients have included use of cryopreserved SC to circumvent the dose-limiting hematologic toxicity of iodine-131–metaiodobenzylguanidine, a targeted radiotherapeutic agent that is able to induce a response in some of these highest risk patients. [201]

### Sarcomas and Other Solid Tumors

Although most children with Ewing's sarcoma and rhabdomyosarcoma can be cured with conventional therapy, the survival of high-risk patients remains poor. The results of autologous HSCT for such patients, including those with metastatic disease at presentation or those with recurrent disease, have been discouraging. [202] For Ewing's sarcoma, the most common myeloablative agent has been high-dose melphalan, either alone or in combination with TBI. Although most patients demonstrate responsive disease, few have a durable remission. The European BMT Solid Tumor Registry, incorporating data from 21 transplant centers, reports an event-free survival of 21% at 5 years for new patients with metastatic disease and 32% for those transplanted in CR2. [203] The results of HSCT for advanced stage or recurrent rhabdomyosarcoma are even less favorable than for Ewing's. HSCT after conditioning regimens based on high-dose alkylator therapy has been well tolerated, but there has been no improvement in DFS. [204,205] Smaller cohorts of patients with better results have been reported. [202,206,207]

Although children with metastatic Wilms' tumor have a high likelihood of cure with conventional therapy, as do those who relapse if their histology is favorable, there remains a small group of patients with adverse features who are rarely cured. The European BMT Solid Tumor Registry reported the outcome of 25 children with Wilms' who were treated with HSCT. [208] Most received melphalan in the conditioning regimen. There was appreciable morbidity and mortality. Those in CR at the time of HSCT had a 50% DFS, although no improvement in outcome was seen for those transplanted with measurable tumor. Gonadal germ cell tumors (GCTs) are particularly uncommon in children, and most can be cured with surgery and conventional chemotherapy. Although there is a significant autologous HSCT experience for adults with GCT, this approach is only applicable to children with poor-risk features at diagnosis, cisplatin-resistant disease, or relapsed disease. Small studies have shown improvement in DFS for these patients using two cycles of high-dose therapy with SC support. Randomized trials are on-going to assess whether the increase in DFS can be replicated in a multi-institutional setting. [209] Relapsed or cisplatin-refractory extragonadal GCTs have a very poor prognosis; although HSCT can result in remissions, the responses have not been durable. [210]

In conclusion, curing children with recurrent or refractory solid tumors remains difficult. [211] It appears that high-dose therapy with HSCT offers improved outcome for some subset of these patients, especially those in CR or with minimal disease at the time of HSCT. Further exploration of rapidly sequenced tandem HSCT using non–cross-reactive agents, the manipulation of SC to reduce tumor contamination, and novel strategies to eliminate minimal residual disease post-HSCT is under way. [200,212]

### Hodgkin's Disease and Non-Hodgkin's Lymphoma

With the exception of Hodgkin's disease (HD) patients who received minimal previous therapy or patients relapsing years after therapy, [213,214] pediatric patients with lymphoma who do not enter remission or who subsequently relapse are rarely cured using therapy at conventional doses. [215,216] and [217] Autologous HSCT has been used to allow dose escalation of a broad range of active agents. Conditioning regimens are alkylator based, with or without the addition of TBI. [218] Among frequently reported regimens are CBV (CY, carmustine, and etoposide), BEAM (carmustine, etoposide, cytosine arabinoside, and melphalan), and BEAC (carmustine, etoposide, cytarabine, and CY). [216] The role of local radiation therapy either before or after HSCT is unclear. [219,220]

Most reports of autologous HSCT for both non-Hodgkin's lymphoma and HD include both adult and pediatric patients. Although overall DFS of 50% is consistently reported, predictive factors related to disease burden and responsiveness to chemotherapy have been identified. Lymphoma patients with favorable characteristics have a DFS of up to 60% after HSCT, whereas less than 20% of poor-risk patients achieve durable remissions. [215,216,219,220] In non-Hodgkin's lymphoma, progressive disease or lack of response to salvage chemotherapy before HSCT is a negative prognostic factor associated with DFS of less than 10%. [219,221] For patients with relapsed HD, bulky disease at the time of HSCT adversely affects outcome [222,223]; response to prior chemotherapy has been shown to impact DFS in some [224,225] and [226] but not all studies. [227] In fact, there is a suggestion that patients who proceed to HSCT in untreated relapse of HD fare best, although the reasons are unclear. [215,228] Patients with HD who are classified as having induction failure or primary progressive disease may be cured by HSCT even in the setting of minimal response to salvage chemotherapy. [229] Whether this represents a response to dose intensification or whether chest x-ray and CT overestimate residual HD is unknown.

Virtually all autologous HSCT for lymphoma now use mobilized PB instead of BM as a hematopoietic SC source. Most reports demonstrate that the time to neutrophil engraftment is shortened and costs reduced, although it has been difficult to show an impact on survival. [25,230,231] Which SC source is preferable in terms of lymphomatous contamination is a complex issue, likely affected by many factors, including the natural history of BM involvement with disease, the degree of BM involvement, and the mechanism by which PBSCs are mobilized. [25,232] No pediatric studies using sensitive techniques, such as PCR, address quantitative differences

in contamination or possible impact on relapse rate. One report found an increased incidence of post-HSCT MDS when PBSCs were used as compared to BM, but this has not been assessed in prospective comparative trials.[233] Overall, the reported risk of developing secondary leukemia and MDS varies greatly, but it is difficult to ascertain the degree to which autologous HSCT increases this risk beyond that conferred by prior exposure to leukemogenic therapy. Some single-institution studies show an actuarial incidence of up to 20% at 10 years post-HSCT with no evidence of a plateau.[16,17,234] The European BMT Lymphoma registry, reporting on 5,000 patients, found an incidence of 4%, similar to that seen after conventional therapy.[235]

The role of allogeneic HSCT has also been investigated, although never in a randomized manner. Most studies use matched sibling donors and suggest a decreased incidence of relapse. This is presumably due to graft-versus-leukemia effect or use of a noncontaminated SC source, or both. There has been no improvement in DFS due to an increased toxic death rate.[227,236,237,238] and [239]

## COMPLICATIONS AFTER STEM CELL TRANSPLANTATION

### Infections

Susceptibility to infection remains a major problem in the management of patients undergoing HSCT. A complete review of the pathophysiology of infectious predisposition in transplant patients, the prophylaxis and treatment of infections associated with HSCT, and post-HSCT immunodeficiency are beyond the scope of this chapter, and only basic principles of infectious management are reviewed here.

Conditioning regimens cause profound myelosuppression, placing patients at risk for bacterial and fungal infections. Mucosal integrity is disturbed and virtually all patients have indwelling catheters, which further increase the risk of bacteremia. Methodologies, such as reverse precautions, hepafiltration, laminar airflow, and handwashing, have all been used to minimize contact of these compromised hosts with infectious agents. The relative contribution of any or all of these maneuvers is unclear. Historically, gram-negative bacteria, known to be virulent pathogens in neutropenic patients, were responsible for much of the morbidity and mortality seen in HSCT. In recent years, gram-positive organisms have emerged as the most common bacterial pathogens.[240] Use of broad-spectrum antibiotics as well as the widespread use of indwelling central venous lines are likely contributors to this change in epidemiology. Fungal infections have also become increasingly problematic. Although HSCT patients should be considered for antifungal prophylaxis, the relative value of prophylaxis versus the risk of generating resistant organisms has not been completely elucidated.[241] Patients also demonstrate deficits in cell-mediated immunity relatively rapidly after the completion of the conditioning regimen. Thus, reactivation of viruses, such as herpes simplex virus I and II and, subsequently, cytomegalovirus, is quite common. The risk for such reactivation is generally defined by recipient seropositivity, and seropositive patients may receive prophylactic antiviral therapy based on institutional practice.

Even after neutrophil recovery and repair of epithelial barriers have taken place, patients appear to remain at significant risk for infection. This risk is particularly marked in patients undergoing allogeneic HSCT in whom depressed cellular and humoral immunity persist for protracted periods. The degree to which host immunity is impaired is influenced by many factors, including patient and donor age, conditioning regimen used, degree of HLA disparity between recipient and donor, presence of acute or chronic GVHD, and type of post-HSCT immunosuppression. Due to this persistent delay in immune recovery, the majority of allogeneic HSCT patients is maintained on prophylaxis against *Pneumocystis carinii* for at least 1 year post-HSCT. Autologous HSCT recipients most often receive prophylaxis for a shorter interval, generally 6 months. The most common post-HSCT infection may be varicella-zoster, although visceral dissemination or mortality in children is rare.[242] Prophylaxis with acyclovir during the months after HSCT may be useful for some patients, although the overall value of this approach has yet to be established. Immune recovery appears to occur more quickly and more completely in children than in adults. This advantage is believed to derive from greater residual thymic function and perhaps to the relatively higher likelihood of young donor age.

### Veno-Occlusive Disease of the Liver

Veno-occlusive disease (VOD) is a clinical diagnosis characterized by painful enlargement of the liver, fluid retention, and jaundice.[243,244] It is the most common serious manifestation of regimen-related toxicity occurring somewhat more frequently after allogeneic than autologous HSCT. The incidence of significant VOD in adults is reported to be between 10% and 20%, with pediatric patients less often affected.[245] The differential diagnosis is broad and includes infection, drug toxicity, and acute GVHD as well as VOD. Ultrasound examination with Doppler flow studies can detect hepatomegaly, ascites, and reversal of blood flow in the portal vein, which are consistent with but not diagnostic of VOD. Damage to the centrilobular area of the liver by high-dose therapy produces a characteristic pathologic appearance, and liver biopsy can provide a definitive diagnosis based on concentric narrowing or fibrous obliteration of terminal hepatic venules and necrosis of centrilobular hepatocytes. In addition to providing tissue, liver biopsy allows the measurement of wedged hepatic venous wedge pressure. An elevated pressure (greater than 10 mm) has approximately 90% specificity and positive predictive value for VOD.[246] For many patients, however, especially those with refractory thrombocytopenia or coagulopathy, or both, this procedure may carry excess risk and treatment is often initiated based on clinical criteria alone. The cornerstone of management is supportive, with meticulous attention to preserving intravascular volume in the face of capillary leak, third-spacing, and hepato-renal physiology. Most patients with mild or moderate VOD will recover, but those with severe VOD have an almost uniformly fatal course.[247] Attempts to prevent VOD have been unsuccessful to date. Although early studies suggested a beneficial effect of ursodeoxycholic acid in high-risk patients[248] a larger multi-institutional study failed to corroborate these findings.[249] The role of vitamin E, an antioxidant, remains speculative at this time.[250] Another approach to decreasing the incidence of VOD involves monitoring levels of potentially hepatotoxic drugs. This has been successfully used with BU, where higher area under the curve of concentration versus time has been associated with an increase in VOD. Measuring BU levels and subsequent dose reduction in patients with elevated area under the curve have resulted in a reduced incidence of VOD.[251] Based on the microthrombi found in hepatic venules on pathologic examination and a physiology consistent with venous outflow obstruction, therapeutic strategies for established VOD aimed at fibrinolysis and anticoagulation have been evaluated. Agents such as tissue plasminogen activator have shown efficacy but have been associated with excessive morbidity from complications related to bleeding.[252] Defibrotide, a single-stranded polydeoxyribonucleotide that decreases thrombin generation and increases fibrinolysis without causing systemic anticoagulation, has been used. No serious hemorrhage occurred, and response rates were encouraging.[253] The role of this agent must be established in additional studies.

### Acute Graft-Versus-Host Disease

Acute GVHD is a clinicopathologic syndrome of enteritis, hepatitis, and dermatitis that develops within 100 days of allogeneic HSCT. The effector cells are thought to be donor T lymphocytes that recognize antigenic disparities between donor and recipient. In addition, the altered host milieu promotes the activation and proliferation of inflammatory cells with resulting dysregulated production of inflammatory cytokines secreted by many cell types in addition to T cells.[254] This cytokine network may be the final common pathway for the tissue damage associated with GVHD, and this entire cascade has been described as a “cytokine storm.”[255,256]

GVHD is staged by the degree of organ involvement, and these stages are summed into an overall grade ( Table 16-5, Table 16-6, and Table 16-7). Several conventions for grading are currently in use. The skin is the organ most frequently involved. Skin GVHD classically manifests as a maculopapular rash that is pink to deep red in color and may be painful. The most severe form of dermal GVHD produces bullae and extensive epidermal separation that resembles toxic epidermal necrolysis. The differential diagnosis of skin rash in this setting is extensive and includes drug allergy, viral exanthem, or reaction to the conditioning regimen, particularly TBI. Differential sensitivity of skin biopsy is not high and is negatively affected by temporal proximity to the conditioning regimen.[257] Histologically, GVHD is associated with basal cell vacuolar degeneration with apoptosis (single-cell necrosis) of epidermal cells and, in severe cases, separation of the dermal/epidermal junction.

| Stage | Skin | Liver | Gut |
|---|---|---|---|
| + | Maculopapular rash: <25% body surface | Bilirubin, 2–3 mg/dL | Diarrhea, <1,000 mL/d<br>Nausea and vomiting |
| ++ | Maculopapular rash: 25–50% body surface | Bilirubin, 3–6 mg/dL | Diarrhea, 1,000–1,500 mL/d<br>Nausea and vomiting |
| +++ | Generalized erythroderma | Bilirubin, 6–15 mg/dL | Diarrhea, >1,500 mL/d<br>Nausea and vomiting |
| ++++ | Desquamation and bullae formation | Bilirubin, >15 mg/dL | Pain or ileus |

**TABLE 16-5. CLINICAL STAGING OF ACUTE GRAFT-VERSUS-HOST DISEASE**

| Grade | Skin | Liver | Gut | Functional impairment |
|---|---|---|---|---|
| 0 (none) | 0 | 0 | 0 | 0 |
| I (mild) | + to ++ | 0 | 0 | 0 |
| II (moderate) | + to +++ | + | + | + |
| III (severe) | ++ to +++ | ++ to ++++ | ++ to +++ | ++ |
| IV (life-threatening) | ++ to ++++ | ++ to ++++ | ++ to ++++ | +++ |

[a]Stages indicated by + signs.
From Thomas ED, Storb R, Clift RA, et al. Bone marrow transplantation. N Engl J Med 1975;16:832–843, with permission.

**TABLE 16-6. OVERALL CLINICAL GRADING OF ACUTE GRAFT-VERSUS-HOST DISEASE (GLUCKSBERG)[a]**

| Grade | Skin Maximum stage | Liver Maximum stage | Gut Maximum stage |
|---|---|---|---|
| 0 (none) | 0 | 0 | 0 |
| A | + | 0 | 0 |
| B | ++ | ++ | ++ |
| C | +++ | +++ | +++ |
| D | ++++ | ++++ | ++++ |

[a]Stages indicated by + signs.
From Rowlings PA, Przepiorka D, Klein JP, et al. IBMTR Severity Index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. Br J Haematol 1997;97:855–864, with permission.

**TABLE 16-7. OVERALL CLINICAL GRADING OF ACUTE GRAFT-VERSUS-HOST DISEASE (INTERNATIONAL BONE MARROW TRANSPLANT REGISTRY)[a]**

Hepatic GVHD usually presents as a cholestatic process with elevation in bilirubin and alkaline phosphatase. Definitive diagnosis is often difficult due to the many possible causes of liver dysfunction in these patients (e.g., infection, drug toxicity, and biliary sludging). On pathologic examination, portal triaditis with bile duct damage is found.[258] GVHD of the lower gastrointestinal tract presents as diarrhea, which can be voluminous. It is often bloody and may be associated with crampy abdominal pain. Edema and mucosal sloughing are seen endoscopically and histology reveals single cell necrosis and, if severe, complete loss of crypts. Allogeneic transplant patients receive prophylaxis to prevent the development of GVHD, either through pre-HSCT graft manipulation, such as TCD, or through administration of post-HSCT immunosuppression. Most studies have demonstrated that the combination of cyclosporine and methotrexate is superior to either agent alone. [259] Optimal drug combinations are the subjects of current studies. TCD has proven an effective mechanism by which to decrease acute GVHD, but associated graft failure, relapse, and post-HSCT lymphoproliferative disorders have made it difficult to demonstrate any impact on overall survival. [260],[261] and [262] Other approaches to GVHD prevention, such as the use of augmented SC dose with exhaustive TCD or induction of host tolerance in the donor, are being explored in clinical trials. [263],[264] If GVHD occurs despite prophylaxis, further immunosuppression is necessary. Addition of or increased doses of steroid is the most common and effective approach. [265] The treatment of steroid refractory GVHD remains problematic despite aggressive interventions, and most patients succumb to infection or organ dysfunction.

### Graft Failure

Graft failure is relatively uncommon after allogeneic HSCT for hematologic malignancies, occurring in approximately 1% of patients receiving SCs from genotypically identical donors[266] and increasing to 5% to 10% if the donor and patient are mismatched. Other risk factors for graft failure include TCD, low marrow nucleated cell dose, and a positive pretransplant crossmatch for antidonor lymphocytotoxic antibodies. [267],[268] The exact mechanism of rejection is not known, but the utility of increased donor cell number or increasing recipient immunosuppression, or both, in the setting of TCD suggest both host and donor elements may be contributory. Once graft failure occurs, treatment with growth factors[269] or re-infusion of donor cells may be successful in a small number of patients. However, mortality remains high due to prolonged neutropenia and consequent infections.

### Late Complications and Sequelae of Stem Cell Transplantation

The type and severity of delayed complications arising after HSCT are related to the underlying disease for which HSCT was performed, the conditioning regimen and SC source used, and the age of the patient at the time of HSCT. Because these late effects are protean and can occur months to years after HSCT, it is important that this group of patients receive lifelong follow-up either at or in concert with a transplant center. As more patients survive HSCT, there is increasing attention being devoted to obviating or ameliorating long-term sequelae. Although these complications can be problematic or even life-threatening for a subset of HSCT survivors, one study assessing a cohort of adult and pediatric patients 5 years after HSCT found that 90% were in good health and had returned to work or school in a full-time capacity.[270]

#### *Chronic Graft-Versus-Host Disease*

Chronic GVHD (CGVHD) is a potential complication of allogeneic HSCT that ranges in impact from trivial to devastating. Major risk factors for the development of CGVHD are prior acute GVHD, donor-recipient HLA disparity, and increasing patient age. [271],[272] and [273] The decreasing incidence of CGVHD in the 1990s reflects more sophisticated HLA matching of unrelated donors as well as more effective strategies for preventing acute GVHD. In the past, CGVHD was significantly less likely to occur with a matched sibling versus an unrelated donor.[274] More recent data show that in the pediatric population there may be little to no significant difference in severe CGVHD rates between sibling and matched unrelated donors.[97] CGVHD primarily affects the same organ systems involved by acute GVHD—skin, liver, and gastrointestinal tract—but may affect others as well. In the skin, manifestations range from dry patches or areas of variegated pigmentation to extensive dermal scarring that produces thickened atrophic skin and joint contractures. Hair follicles can be involved, with consequent alopecia over part or all of the scalp as well as loss of hair on the arms and legs. Other skin appendages may also be lost, leading to dry skin and limited ability to sweat. Changes in the upper and lower gastrointestinal tract may be observed. Patients may have depapillation of the tongue and scarring of the buccal mucosa. The lips may develop variegated coloration and blurring of the vermilion border. Sclerodermatous changes of the mucous membranes and salivary glands can produce xerostomia. In addition to causing patient discomfort, these conditions have consequences that include increased risk of dental caries and gingival injury. [275] Patients with CGVHD are often underweight. This can be a result of anorexia, esophageal webs, or strictures that make swallowing difficult or chronic diarrhea with malabsorption. A careful history can narrow the differential diagnosis, but endoscopy with appropriate biopsies may be necessary. CGVHD of the liver usually presents as a cholestatic process with elevation in alkaline phosphatase. Elevation in bilirubin and liver enzymes can also occur with findings that can progress to a syndrome similar to primary biliary cirrhosis. Pulmonary dysfunction can also occur in the setting of CGVHD. The most frequent clinical presentations of pulmonary disease are nonproductive cough, wheezing, and dyspnea, although children with significant defects can also be completely asymptomatic. Pulmonary function tests should be obtained regularly post-HSCT, particularly in patients with CGVHD or in the presence of persistent pulmonary symptoms. Most patients have obstructive defects with interstitial fibrosis and bronchiolar changes seen on biopsy. This complication may occur more frequently in children, with one study reporting an incidence of 26%. [276] Restrictive defects, though less common, can occur as well.[277]

The most effective therapy for CGVHD is unknown.[278] As the effector mechanisms of CGVHD are poorly understood, current modalities are directed at immunosuppression of donor T lymphocytes, the effector cells of acute GVHD. Initial therapy usually includes steroids, calcineurin inhibitors, such as cyclosporin and FK 506, and/or antithymocyte globulin.[279] Newer modalities include monoclonal antibodies directed against activated T cells [280] as well as alternative immunosuppressants such as mycophenolate.[281,282] Children with CGVHD are profoundly immunocompromised, both by their underlying immune dysregulation and its pharmacologic management, and require continuing prophylaxis against opportunistic infections and aggressive evaluation of fever. Infectious death is common, often from encapsulated organisms. A proportion of patients are functionally asplenic. [283] Antibacterial prophylaxis is indicated. Despite advances in supportive care, CGVHD remains a major cause of morbidity and mortality for children undergoing allogeneic HSCT.

### *Secondary Malignancies*

After HSCT, patients are at risk for a variety of secondary neoplasms. Secondary MDS/leukemia, presumed to arise from prior exposure of SCs to chemotherapeutic agents or irradiation, or both, is a significant problem after autologous HSCT. Risk factors include prior therapy with topoisomerase II inhibitors or high cumulative doses of alkylating agents, older age at HSCT, and poor BM function before transplant. [284] The incidence of therapy-induced MDS has been reported to range from 4% to 20% at 5 to 6 years after autologous HSCT.[16,285] Posttransplantation lymphoproliferative disease (PTLD) is another significant source of malignancy after HSCT. The use of either TCD or antithymocyte globulin for prevention or treatment of GVHD is associated with PTLD [286] presumably because of the greater degree of ensuing immunologic dysfunction. Patients receiving transplants from mismatched donors, those with CGVHD, and those transplanted for congenital immunodeficiencies are also at increased risk.[287,288] PTLD is usually but not always Epstein-Barr virus (EBV)–positive and can present as an infectious mononucleosis syndrome, as a localized process, or as a fulminant lymphoma. The abnormal cells may be polyclonal, oligoclonal, or monoclonal, with some suggestion that response to therapy is inversely related to the degree of clonality. [289] The treatment depends on the extent of disease and may include decreasing immunosuppression, treatment with interferon, or chemotherapy. Other strategies have involved prophylactic or therapeutic infusions of EBV-specific autologous or allogeneic donor T lymphocytes.[290,291] This therapy has been effective, but requires purified and expanded donor cells to be available and can carry a significant risk of GVHD. In addition, not all donors are EBV seropositive, limiting the ability to derive EBV-specific lines or clones. More recently, the use of monoclonal anti-CD20 antibody IDEC-C2B8 (rituximab) has been successfully employed.[292]

Last, patients post-HSCT may develop solid tumors. Those receiving TBI in the conditioning regimen appear to be most vulnerable. Tumors include skin and soft tissue cancers, with a preponderance of tumors localized to brain, thyroid, and salivary glands. The majority of skin neoplasms are basal cell or squamous cell carcinomas, although melanoma can also occur.[293] Bone tumors may develop and can be benign (osteochondromas)[294] or malignant (osteosarcoma). In contrast to PTLD, which generally presents within the first 6 months after transplantation, solid tumors occur later, at a median of 4 years post-HSCT. The overall incidence of malignant neoplasms is approximately 2%, which is eight times greater than expected in an age-matched cohort. [293] However, a recent report found a much higher incidence in children undergoing allogeneic HSCT for acute leukemia. [288] The cumulative risk of solid tumors was 11% at 15 years post-HSCT. Children younger than 5 years of age at the time of HSCT were at greatest risk. Prior cranial radiation therapy for CNS prophylaxis was associated with a particular increase in the incidence of brain or thyroid cancer. HD is also more frequent after allogeneic HSCT, occurring at a median of 4 years post-HSCT. [295] Reported patients all had acute or CGVHD, or both, implicating immune dysregulation as a potentiating factor.

### *Endocrine Complications*

Endocrine disturbances after HSCT are myriad. Primary gonadal failure has been described in approximately three-fourths of post-pubertal females after treatment with TBI-containing conditioning regimens, but can also occur after BU/CY or other chemotherapy-only conditioning. [296,297 and 298] Less longitudinal data are available for girls who were pre-pubertal at the time of HSCT, but it appears that the majority has experienced complete ovarian failure. [299,300 and 301] Whether this will be true for patients receiving fractionated or hyperfractionated TBI, or both, or other novel preparatory regimens remains to be established. Regular follow-up is essential so that puberty can be chemically induced and the ill effects of low estrogen production, such as osteoporosis, avoided by appropriate supplementation. Although Leydig cell function and testosterone production are usually unimpaired, [298,300,302] virtually all male patients appear to be infertile after the use of TBI or myeloablative chemotherapy. Sperm banking should be addressed with all eligible males before conditioning, and the transplant community should remain alert to advances in sperm and oocyte collection and preservation techniques. Patients have occasionally preserved their reproductive function, particularly those who are pre-pubertal at the time of HSCT, so subsequent evaluation is important. For women who do conceive after HSCT, there is an increased chance of having an infant with low birth weight and an increased rate of spontaneous abortion for those who have received TBI. There does not appear to be any increased risk of congenital anomalies in children born to survivors of HSCT.[296]

Thyroid dysfunction is well documented after HSCT and is most prevalent after regimens containing TBI [298] but can occur after chemotherapy-only conditioning regimens as well.[303] The use of fractionated TBI has decreased the incidence of hypothyroidism from more than 30% to 10%. [302,304] Thyroid function tests should be checked annually, as it may take many years for thyroid abnormalities to present. Treatment of thyroid hormone deficiencies allows for optimum growth as well as decreases the risk of thyroid malignancy.

### *Growth and Development Complications*

Children undergoing HSCT are at risk for growth failure. Patients who receive TBI after prior cranial irradiation are at greatest risk. [305] Reports on the linear growth of children after BU-based conditioning are conflicting. Some studies show no adverse effect on final height, [305,306] whereas some show effects comparable to TBI-based regimens.[307] Prolonged steroid use post-HSCT can further contribute to decreased growth rate. [307] Cognitive outcome for children after HSCT has not been systematically and extensively investigated, but children older than 6 years old at HSCT appear to have minimal risk of neurocognitive sequelae. Younger children, in particular those younger than 3 years old, show some small risk of cognitive decline when tested 1 year post-HSCT. [308] This decline does not seem to be progressive, as cognitive ability stabilized on follow-up evaluations. [309]

### *Other Late Complications*

A substantial risk of cataract development exists for patients receiving TBI. Fractionation of the TBI dose decreases the risk from 60% to 10% to 30%. [310,311] As cataracts typically develop years after HSCT, annual ophthalmologic evaluation is indicated. Patients may also have decreased lacrimation secondary to chronic GVHD or chemoradiotherapy that can result in keratopathy. The most important bony complication of HSCT is avascular necrosis. [312] Patients may develop findings of renal disturbance similar to classic hemolytic-uremic syndrome. The disorder usually presents as moderate hemolysis and renal insufficiency beginning 4 to 9 months after HSCT. The mechanism appears to be radiation-induced damage to renal arterioles and capillaries with secondary effects on glomeruli and tubules. Most cases resolve spontaneously over time with resolution of clinically apparent hemolysis, although subtle deficiencies in renal function may be more persistent. [313] Hemolysis post-HSCT can also be a result of ABO incompatibility between donor and patient because inheritance of blood group antigens is independent of the HLA gene complex.[314] This does not usually present a clinical problem. Red cell depletion of the infused BM prevents significant acute hemolysis. A short delay in reconstitution of the red cell compartment occurs, but prolonged red cell aplasia, although reported, is uncommon. [315] Autoimmune hemolytic anemia may also result as a consequence of HSCT-associated immune dysregulation.[316,317] Immune-mediated cytopenias have been reported to involve the other cell lines as well. [318,319] These cytopenias can occur months to years after allogeneic HSCT and are most often reported in the setting of TCD or with the use of alternative donors, but can be seen even after autologous HSCT. This can be a pernicious problem that may require prolonged periods of immunosuppression with frequent relapses and can be fatal.

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# 17

# GENE TRANSFER AND THE TREATMENT OF PEDIATRIC MALIGNANCY

MALCOLM K. BRENNER



## INTRODUCTION

It has been asserted that every century has its revolution in medicine—public health in the eighteenth century, surgery in the nineteenth century, and antibiotics in the twentieth century—and that the ability to transfer and express new genes in cells will fulfill this role in the new millennium. Others counter by pointing out that because genetic disorders account for less than 2% of all human disease, even fully evolved forms of gene therapy can have only a limited impact on human health. The truth, as always, lies somewhere in between. Although only a few diseases are caused by single gene defects, a far larger proportion of human ills, including cancer, can be attributed to the interaction of multiple genes and gene defects. More important, however, is that the correction of genetic defects is only one of many potential clinical applications of gene transfer. Of greater probable importance will be the use of gene transfer to act as a form of drug delivery system, as a means to modify the behavior of cells, by ablating normal characteristics or adding new ones, and as a way to mark cells so their behavior and fate can be monitored *in vivo*. When one considers these possibilities, it becomes evident that gene transfer may have a role in most human disease. Achieving these goals will require substantial improvements in current gene transfer technologies, however. For example, many applications require genes to be transferred with high efficiency and precise targeting. Targeting does not end with the penetration of the cell membrane, because the transferred genetic material must be targeted to the nucleus. If long-term expression is needed, the transferred DNA usually has to integrate, ideally in a specific site in the DNA. Finally, the transferred genes should respond to appropriate cellular regulatory mechanisms so the gene product will be produced in correct quantities and at appropriate times. We are currently a long way from achieving any of these aims with current methods of gene transfer. To date, therefore, the practice of human gene therapy has largely been governed by a need to match the desires of the investigator with the capabilities of the available transfer technology. These limitations notwithstanding, more than 100 clinical protocols for gene transfer in cancer have already been approved. Early clinical results have often been of both scientific and clinical value and have indicated the considerable potential of the approach.

They have also demonstrated, however, that the highest standards of clinical practice have not always been adhered to. As with any new field, regulatory and public scrutiny of gene therapy studies has been intense and has revealed a need for improved training of investigators and superior record keeping. The ensuing tumult has undoubtedly slowed progress toward gene therapy trials as institutions begin to remedy the deficits in their regulatory and data handling infrastructure. Longer term, however, it is likely that the presence of chastened but more experienced clinical investigators implementing better designed and better run studies will prove of benefit not just to gene therapy but to clinical investigation as a whole.

This chapter reviews the successes, limitations, and long-term potential of gene transfer techniques for therapy of pediatric malignancy, and reviews the accomplishments in the field as well as the impediments to progress. Most important, this chapter gives an idea of the incremental way in which gene transfer technologies will supplement, long before they supplant, current therapeutic approaches to these cancers.

There are four major approaches to incorporating gene transfer into therapy for childhood cancer. [1] The tumor cell itself is modified, either by “repairing” one or more of the genetic defects associated with the malignant process, by introducing a gene that triggers an antitumor immune response, or by delivering a prodrug-metabolizing enzyme that renders the tumor sensitive to the corresponding cytotoxic agent. [2] The immune response to the tumor is modified by altering the specificity or effector function of immune system cells.[3] The drug sensitivity of normal host tissues is decreased by delivering cytotoxic drug-resistance genes to marrow precursor cells, thereby increasing the therapeutic index of chemotherapy. [4] Finally, it is possible to mark normal or malignant cells so that the efficacy of conventional therapies can be monitored more closely. These efforts must all be tempered by the limitations of current vectors, which invariably lack the efficiency and targeting capacity that are required for optimum results.

## CURRENT VECTORS

Because no single vector has all the characteristics desired for effective gene transfer, the choice of a gene transfer agent requires the demands of the specific application to be matched as closely as possible with the characteristics of the vector. This section outlines the structure and function of the most widely used gene transfer methodologies currently in clinical practice: retroviruses, [1,2] and [3] adenoviruses,[4,5] adeno-associated virus (AAV),[6] and liposomes.[7]

### Retroviruses

Figure 17-1A shows the structure of a classic retroviral vector.[8] The structural and replicative genes (gag, pol, and env) of a murine retrovirus are replaced by one or more genes of interest, driven either by the retroviral promoter in the 5' long terminal repeat or by an internal promoter. The retroviral constructs are made in cell lines in which the missing retroviral genes are present *in trans* and thus reproduce and package a vector that is not replication competent. Retroviral vectors have a wide target cell range, and the genetic information they convey is integrated into the host cell DNA. Thus, the transferred gene not only survives for the entire life-span of the transduced cell, but is also present in that cell's progeny. Hence, these vectors are ideal for transferring genes into rapidly dividing cell populations, such as hemopoietic stem cells or lymphocytes. Provided that replication-competent virus is absent, the vector preparations appear to be nontoxic. However, retroviral vectors have several disadvantages. Because expression of the transferred gene requires viral integration of the genome, and hence a population of dividing cells, the efficiency of transfer to many types of cell may be low.[3] It is possible to increase efficiency by bringing vector particle and target cell into close physical apposition, for example, by performing transduction on substances like fibronectin, but even then, overall levels of transfer leave much to be desired. Furthermore, because the integration events themselves occur largely at random in the host cell DNA, regulatory genes could conceivably be damaged, contributing to oncogenesis. [9] Finally, retroviral vectors are not well suited for use *in vivo*,[10] because they are generally unstable in primates, and, as yet, they cannot be targeted to specific cell types. The development of pseudotyped particles, in which a retroviral vector genome is incorporated into an envelope derived all or in part from a different virus, may improve the *in vivo* stability of retroviral vectors and alter their target cell range.

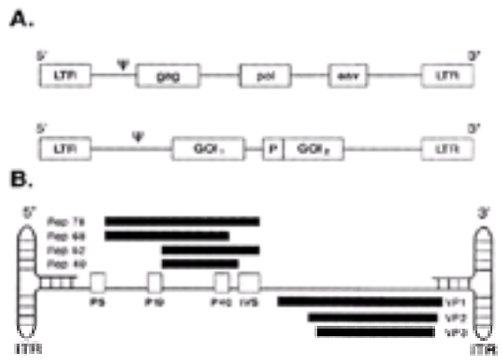


**FIGURE 17-1. A:** Retrovirus and retrovirus vector. The reverse transcriptase (gag), polymerase (pol), and envelope (env) coding sequences are removed and are supplied *in trans* by a producer cell. One or more genes of interest (GO1) are inserted, driven from the viral long terminal repeat (LTR) promoter or from an internal promoter (P). The viral packaging signal (y) remains in the vector, so it is appropriately packaged by the producer cell. **B:** Structure of adeno-associated virus (AAV). The AAV genome is a linear single-stranded DNA molecule. The viral genome is transcribed in three overlapping regions producing seven primary transcripts. The transcripts obtained from each gene are shown as black lines. The virus has two palindromic inverted terminal repeats (ITR) that, in combination with products of the rep region, are responsible for site-specific integration. The rep products are also required for replication during coinfection with adenovirus. VP1 to VP3 encode the viral capsid proteins. Promoter regions for these genes are boxed (p5, p19, p40, IVS).

### Adenoviruses

First generation adenoviral vectors are E1 (early protein) deletion mutants and therefore are not replication competent. [4,5] These vectors infect a wide range of cell types and, unlike retroviruses, can transfer genes into nondividing cells. The vectors are reasonably stable *in vivo* and can be used to infect cells *in situ*. Examples include gene transfer into respiratory epithelium (the CFTR gene in cystic fibrosis [4]) or liver (genes encoding factor VIII and factor IX in hemophilia A or B [11]). Adenoviral vectors are generally nonintegrating, however, so that the gene products are expressed from episomal DNA. [12] The episome is often lost after cell division and can be inactivated or lost even in a nondividing cell. [11] Thus, adenoviral vectors are unsuited for any application that requires long-term expression in a rapidly turning-over cell population or transfer into a stem cell and expression in that cell's progeny. A more important limitation is that most adenoviral vectors are immunogenic. Immune responses are generated against the vector proteins themselves (often preventing readministration of the vector), as well as against low levels of adenoviral proteins, expressed even when cells are transduced by defective viruses. [11] Moreover, concurrent expression of adenoviral genes appears to increase the probability of developing an immune response to the transgene product. The mechanism for this effect is unclear, but it is likely related to the ability of adenoviral entry into many cell types to trigger the release of cytokines, such as interleukins (ILs) 6 and 8, which also induce a potentially highly destructive local inflammatory response.[13] Finally, adenoviral damage to vascular endothelium and to other organ systems may cause a potentially lethal disseminated intravascular coagulopathy, or a more chronic hepatocyte hypertrophy and hepatic fibrosis. [14,15] As deletion of the E1 region alone does not prevent these toxicities, subsequent generations of adenovectors have been prepared that lack more than one set of adenoviral genes (e.g., *E1* and *E4*,[16] *E2a*[17] and *E4*,[18] or *E1* and *E3*[19,20]) (reviewed in Hitt et al.[21]). Although showing less immunogenicity and toxicity and more durable transgene expression in some studies, such modified vectors produced little or no benefit in others.[16,17,18,19 and 20] Indeed, administration of an *E1*/*E4*-deleted vector has been linked to a human death. [22]

The quintessential attenuated adenovector is the so-called helper-dependent or “gutless” vector, in which virtually all of the adenoviral genes have been removed and replaced with the gene of interest and its promoter, together with irrelevant DNA to allow packaging in the viral envelope. [23] These vectors can only be made with the assistance of a helper adenovector, which must then be separated from the deleted vector. [24] Helper-dependent vectors have shown a much higher therapeutic index than conventional adenovectors in several different models. [21,23,25,26 and 27] Importantly, they also seem to be much less immunogenic, so that transduced postmitotic cells (e.g., muscle or liver) may secrete vector-derived proteins over many months, [16,28] a prime consideration in the treatment of many deficiency disorders.

### Adeno-Associated Vector

AAV[6] (Fig. 17-1B) is a “dependovirus” that can replicate only when an AAV-infected cell is coinfected with adenovirus or herpesvirus. Structurally, AAV is a DNA parvovirus, containing two palindromic inverted terminal repeats. When linked with two gene products from the rep gene region, these repeats favor site-specific integration of chromosome 19[29] by the AAV. Thus, AAV, like retroviruses, should persist for the entire life of the host cell and its progeny. Because genomic integration by AAV is relatively site specific, the risk of oncogenesis is low. Although, in contrast to retroviruses, AAV may integrate the genomes of nondividing cells and become permanently expressed even in resting or postmitotic cells, this and other putative advantages have proved hard to exploit in clinical practice. For example, the rep gene products that contribute to site-specific integration are toxic to virus-producing cells and are usually deleted from vectors. However, rep-deficient vectors appear to lose almost all of their ability to integrate genomes, regardless of site. [6] Finally, it has proved difficult to develop high-titer producer cell lines, free of contaminating helper adenoviruses. Although the introduction of AAV into clinical use has therefore been delayed, preliminary studies in which AAV vectors encoding factor IX are injected into postmitotic tissues, such as muscle, are already producing encouraging results. [30]

### Liposomes and Other Physical Methods

Clinical experience with the available physical methods of gene transfer has primarily involved cationic liposome/DNA complexes, [7,31,32] which fuse with the cell membrane and enter the endosomal uptake pathway. DNA released from these endosomes may then pass through the nuclear membrane and be expressed. The main advantage of liposomes is that they are nontoxic and can be given repeatedly. In some cell types, high levels of gene transfer have been obtained by this method.[7] Liposomes are rather unstable *in vivo*, but liposomal transfer by local injection of human melanoma cells *in situ* has resulted in the expression of a new gene (HLA-B7).[7] However, the DNA transferred by liposomes does not integrate the genome, and despite the incorporation of a variety of ligands into the liposome-DNA complex[32] the ability to target these vectors is still quite limited. More recently, successful gene transfer *in vivo* has been reported with use of a bio-ballistic (“gene gun”) technique in which DNA coated onto colloidal gold particles is driven at high velocity by gas pressure into the cell. It is not yet clear whether this approach can be used to transduce normal or malignant hemopoietic progenitor cells with sufficient efficiency to allow therapeutic application. Localized electroporation of plasmid DNA using microelectrodes has also proved to be highly effective for the transduction of muscle and skin and can be followed by significant transgene expression.

### Other Vectors

Other vectors, including herpesviruses[33] and lentiviruses,[34] have been proposed as high-efficiency transducers of many cell types, and herpesviruses have recently entered clinical trials. However, although these viruses may become future substitutes for currently available vector systems, most investigators now accept that no naturally occurring virus and no simple physical vector will ever prove suitable for all gene therapy purposes. Ultimately, therefore, entirely new synthetic or semisynthetic vectors will have to be developed.[35] Possibilities include the generation of hybrid viral vectors, which may combine, for example, the *in vivo* stability of adenoviruses and the integrating capacity of retroviruses. Alternative, fully synthetic vectors will be developed by combining components from multiple different vectors, allowing safe, efficient, and specific gene transfer and regulation. In the meantime, gene therapy protocols for immunodeficiency and for cancer will require investigators to circumvent the limitations of current vectors and to choose their agents on the basis of the most important feature required. For example, a requirement for long-term gene expression by the progeny of hemopoietic stem cells dictates a retroviral vector, whereas a protocol specifying transient expression of differentiated malignant cells and their transduction *in vivo* would favor adenoviral vectors.

## IMPROVING VECTOR SYSTEMS

### Targeting

To target viral vectors to specific cells or organ systems, it is usually necessary not only to add a targeting ligand to provide the new specificity, but also to disrupt preexisting ligands so that the new specificity replaces, rather than adds to, the old. Moreover, the new ligands should allow the virus to enter the cells by membrane fusion or active transport, through the same intracellular pathway as the native vector. For adenoviruses in particular, retargeting may also be an important means of

limiting virus-induced toxicity to vulnerable organs. Adenoviruses bind to at least two molecules on their target cells—the *Coxsackie* adenovirus receptor and cell surface integrins (usually av3 or av5).[21,36,37 and 38] Binding is mediated by domains on the adenoviral knob protein. Because the sequence and crystal structure of this protein is known, one can identify new ligand sequences and incorporate them into positions that disrupt preexisting patterns of binding and establish new ones. However, the complexity of the process has hampered efforts to effectively retarget viral vectors. Retargeting may be much simpler with liposomal vectors, whose intrinsic targeting capabilities are limited, so that simple addition of a ligand could secure the desired effect. Despite the appeal of these retargeting strategies, the only success reported to date has been infection of liver cells through directed binding to the asialoglycoprotein receptor.[39]

### Regulation of Transgene Expression

Most effective gene therapies require regulation of the transgene. Two approaches are available. The first relies on endogenous regulatory elements by replacing the defective sequence with an inserted wild type sequence—the process of homologous recombination. One promising technique for achieving this effect is the so-called chimeroplast technology,[37,38] which utilizes a partial sequence of the gene of interest, incorporating the corrected base pair attached by hairpin structures at each end to RNA complementary to the complementary DNA strand sequence.[40] An additional short DNA sequence is included at the mid-point of the RNA. This chimeric molecule is resistant for 48 hours to intracellular exonucleases or ribonucleases. These chimeroplasts appear particularly effective for repairing single nucleotide defects, but may be less effective whenever more lengthy sequences are abnormal or absent. Nonetheless, in animal models of several diseases (e.g., Crigler-Najjar disease or uridine diphosphate-glucoronysyl transferase deficiency,[32] or $a_1$-antitrypsin inhibitor deficiency and the factor IX deficiency of hemophilia B[41]), as many as 40% of the hepatic cells can be repaired *in vivo*, and clinical trials of this approach are imminent.

For most disorders, regulatory elements may need to be introduced with the transgene. Thus, several regulatory structures are being developed with the intention of using orally absorbed small molecules to control transgene expression. The three systems closest to clinical use are regulated by rapamycin or tetracycline and their analogues,[42] or by the antiprogestin agent RU486.[43] By modifying the DNA-binding domains, it is possible to alter the target sequence that is bound, allowing a repressor and an inducer to be present in the same cell. Similarly, by modifying the receptor for the small molecule, it is possible to use two different oral agents, one to upregulate and one to downregulate production. This provides a lower background than an inducible system alone and a higher level of maximum expression than a repressor alone. Alternatively, the system could be used to turn on two separate genes.

## APPROACHES TO GENE TRANSFER IN PEDIATRIC MALIGNANCY

### Modification of the Tumor

#### *Tumor Correction*

There is an attractive elegance to the strategy of introducing genetic material into a pediatric malignancy to correct the specific genetic defects contributing to the neoplastic phenotype. A number of mutant oncogenes and fusion transcripts have been identified in childhood cancers that are certainly specific to the malignant clone and frequently form a critical component of the malignant process. This approach is technologically demanding, however, as all malignancies result from a multiplicity of genetic abnormalities. Unless correction of a single defect is subsequently lethal to the malignant cell, transfer of an individual corrective gene to a patient with $10^{11}$ or $10^{12}$ tumor blasts will leave a multiplicity of premalignant cells, with a high risk of later transformation. Present methods of gene transfer are also inefficient.[3,44,45 and 46] Even if it were possible to transfer genes to 90% or more of malignant cells *in vivo*—a feat currently beyond any available vector[47]—it would be insufficient to produce more than transient clinical benefit in most pediatric malignancies. Moreover, many relevant gene defects produce molecules with "transdominant" effects that continue to produce a malignant phenotype, even if a wild-type gene is introduced.

Transdominant malignant genes could be neutralized only by ribozymes, by antisense RNA, by intracellular antibody genes (intrabodies), or by homologous recombination with a wild-type gene.[48,49] These "subtractive" approaches to gene transfer are designed to destroy the function of an expressed gene rather than to add a new activity. Ribozymes are RNA structures that are able to cleave specific sequences in the targeted messenger RNA molecules. For clinical use, ribozymes that form hairpin or hammerhead structures are preferred because of their stability, even in the absence of substrate, and their activity under physiologic conditions. The original function of these molecules in viruses and other microorganisms is probably to autocatalyze their own cleavage into functional RNA, and perhaps also to destroy the RNA of invading organisms. For applications in gene transfer, ribozymes may be used to destroy transcripts originating from the unwanted host cell DNA sequence while leaving intact the messenger RNA originating from the transgene. If necessary, the sequences for these ribozymes could be delivered in the same vector as the corrective transgene. Alternatively, chemically modified ribozymes may be administered as drugs. Antisense RNA molecules consist of sequences that bind the sense transcript of interest and prevent translation. These molecules are usually stabilized by the addition of residues that prevent their degradation by host cell ribonucleases, and are usually delivered directly or packaged in liposomes. Although both approaches have the attraction of extreme specificity, in practice it has proved difficult to ensure that expression of ribosomal or antisense genes will be adequate to overcome transdominant effects. There also have been instances in which the specificity of the approach has been less than expected. The "intrabody" strategy is based on the ability to genetically manipulate genes encoding antibodies, so that the antigen-binding domain is expressed intracellularly.[50] Such intrabodies may block protein-protein interactions, alter protein function, or divert proteins from their normal cellular compartment. For example, the a unit of the IL-2 receptor, which is overexpressed in some T-cell leukemias, can be blocked in this fashion *in vivo*.[51] Finally, homologous recombination attempts to replace the defective sequence within the gene with a normal sequence. Until recently, the efficiency of this strategy was far too low to permit its application to human disease. Claims that hybrid RNA-DNA molecules joined by a hinge region may be much more effective at producing recombination, at least over short stretches of DNA, has led to renewed interest in the approach. Plans are well advanced for the treatment of abnormalities such as Crigler-Najjar syndrome,[52] and application to treatment of sickle cell disease[40] and perhaps pediatric malignancy may follow.

The preceding limitations notwithstanding, several tumor correction protocols have been proposed. In spite of the polygenic origins of cancer, it is hoped that certain individual genetic abnormalities will be both pivotal to the malignant process and amenable to correction. For example, efforts are being made to neutralize fusion transcripts such as *BCR-ABL* or activated oncogenes such as *MYB* (in chronic myeloid leukemia), using ribozymes, antisense RNA, or wild-type genes.[53,54 and 55] These efforts have been lent support by success using specific small molecule inhibitors of the enzymes produced by such mutations. Similarly, nonfunctional antioncogenes such as p53 may be replaced by wild-type genes in patients with acute myeloid leukemia or myelodysplasia.[56] Interest is also increasing in targeting the gene pathways involved in regulating apoptosis. Experimental models suggest that even minor perturbations in these pathways can greatly modify the sensitivity of cancer cells to chemotherapy. Finally, it has been suggested that tumor correction may best be used in a preventive manner for patients in whom known single gene defects predispose to subsequent mutagenesis and cancer.

#### *Prodrug-Metabolizing Enzymes*

Efforts have also been made to insert genes that will encode enzymes able to convert harmless prodrugs into lethal cytotoxins. More than a dozen prodrug metabolizing enzyme (PDME) systems have been described. Of these, the thymidine kinase gene (phosphorylates acyclovir/valacyclovir/ganciclovir to toxic nucleoside) has been the most widely used. Other systems in various stages of clinical development include cytosine deaminase,[57,58] which converts 5-fluorocytosine to 5-fluorouracil, the P450-2BI system (converts cyclophosphamide to 4-hydroperoxycyclophosphamide),[59] and the bacterial nitroreductase system[60] (reduces CB 1954 to the more active 4-hydroxylamine). For the PDME approach to be selective for a given leukemia, either the vector or the prodrug product must be targeted to the malignant cell. The first clinical studies to test this novel strategy have aimed for both types of selectivity by introducing a thymidine kinase gene into a tumor cell with use of a retroviral vector.[61,62] On exposure to ganciclovir, the transduced cells phosphorylate the drug. If the cell then divides, the product is incorporated into DNA with lethal consequences, whereas nondividing cells are unaffected. Initial therapeutic study of thymidine kinase gene transfer was made in patients with primary or secondary brain tumors; in this context, there is a particularly clear distinction between tumor cells (which divide and are destined to be killed by the ganciclovir) and normal neurons (which do not divide and should escape unharmed). Retroviral vectors offer additional tumor specificity in this system because they function only in dividing cells, and therefore do not transduce normal neurons.

Transfer of prodrug-metabolizing genes may not require all the tumor cells to be transduced for benefit to be seen. One of the most puzzling features of the original thymidine kinase-retrovirus system was that it worked so well in many preclinical tumor models. Even when fewer than 10% of tumor cells were transduced, ganciclovir destroyed nearly 100% of the tumor cell population.[61,63] This advantage over the tumor correction protocols described earlier appears due to a "bystander" effect. That is, cells that lack the PDME gene can be killed if they are adjacent to transduced cells. The bystander effect is most evident in tumor cells that have gap junctions, so it likely represents the transfer of a toxic metabolite or an apoptotic signal.[63,64] The potency of this effect will likely be much lower among the "gapless" cells of hematologic malignancies. However, an immunologic bystander effect might also occur *in vivo*: Once the tumor cell is killed by the toxic metabolite and is processed and presented by antigen-presenting cells, the host may be immunized against tumor development[35,36] (see following section). At present, retinoblastoma

is the only pediatric malignancy to be treated by this approach. [127]

### *Generation of Tumor Vaccines*

In an attempt to enhance immune recognition of poorly immunogenic tumors, investigators have evaluated the effect of transducing tumor cells with lymphotactic chemokines,[65] cytokine genes,[66] allogeneic major histocompatibility complex (MHC) molecules,[7] or costimulatory molecules such as B7.1 or CD40 ligand[47,67] that activate cytotoxic T cells after engaging their surface ligands or counter receptors.

In murine model systems, the transfection of tumor cell lines with these molecules has augmented immunogenicity. Injection of neoplastic cells in doses that would normally establish a tumor instead recruits immune system effector cells and eradicates injected tumor cells. Often, the animal is then resistant to challenges by further local injections of nontransduced parental tumor. The transduced tumor has therefore acted like a vaccine. In some models, established, nontransduced, parental malignant cells are also eradicated.

There are two major problems in translating these approaches to pediatric malignancies. The primary malignant cells of many pediatric malignancies are highly resistant to transduction by many currently available vectors. This problem may be overcome *ex vivo* by using herpes viruses[33] or modified adenoviral vectors.[68] If adequate transduction of tumor cells cannot be obtained, nontransduced primary tumor cells may be combined with transduced fibroblasts expressing the immunostimulatory genes of interest. Although this approach has proved effective in animal models, it has not yet been validated clinically. A more fundamental concern about the tumor vaccine approach is that neoplastic cells show considerable phenotypic heterogeneity. Moreover, malignant “stem cells” may be phenotypically and functionally distinct from the bulk tumor population,[128] so that a vaccine made from a small proportion of these cells obtained from one site may not express the full array of antigens present in the patient as a whole.

As of this writing, tumor vaccines were being evaluated in more than 200 different clinical trials. Relatively few studies included pediatric patients. Preliminary results in melanoma, renal cell carcinoma, and neuroblastoma suggest that tumor cells transduced with the *IL-2*, *GM-CSF,* or *HLA-B7* gene can be given safely and will frequently produce immunomodulatory effects, including peripheral blood eosinophilia, a rise in natural killer (NK) and activated killer (AK) cell number and activity, and an increase in tumor-specific cytotoxic T-lymphocyte precursor frequency.[69,70,71,72,73] and [74] There have been reports of clinical responses in distal tumor sites, although other metastases have continued to grow (perhaps because their phenotypic heterogeneity allowed them to evade the immune system, as described earlier in this section). There has also been concern that the immune response itself can produce adverse hypersensitivity reaction. and certainly no patients have yet been cured of their disease.[75]

As more information is gained about the safety and efficiency of tumor vaccines, the approach has been altered. Vaccines are beginning to be used as adjuvants to prevent relapse in patients with presumed minimal residual disease, and several different immunostimulatory genes are being introduced in combination into the tumor. The immune response has at least four distinct components: (a) processing of antigen, (b) attraction of lymphocytes to the site of antigen presentation, (c) costimulation of cells that have engaged their antigen-specific receptor and of costimulator molecules, and (d) amplification of the attracted, stimulated cells by growth factors. Immunotherapy that combines two or more of these separate classes of stimulatory agents will likely prove more effective than treatment based on single immunogens. For example, in murine studies, combinations of the T-cell–attracting chemokine lymphotactin, and the T-cell growth factor IL-2 caused regression of preestablished leukemia and neuroblastoma when either agent alone was inadequate.[65]

Studies using combinations of, for example, IL-2 and lymphotactin and of IL-2 and CD40 ligand expressing neuroblastoma and acute leukemia cells have begun, and the increasing availability of vectors able to transduce primary human leukemic cells with high efficiency should simplify exploration of this combinatorial approach.

## Modification of Host Immune System

### *Gene-Modified Cytotoxic T Cells*

A considerable body of evidence indicates that the immune system has the potential to eradicate leukemia and perhaps lymphoma and certain pediatric solid tumors as well.[76,77] and [78] This effect is clearest in patients who have received bone marrow allografts for the treatment of a hematologic malignancy. In those cases, the presence of graft-versus-host disease (GVHD) lowers the risk of subsequent relapse, whereas measures that prevent GVHD, such as T-cell depletion or the use of an identical twin allograft, are associated with an increased risk of disease recurrence. This so-called graft-versus-tumor effect may simply be another manifestation of GVHD in which both normal and malignant host cells share the same host-specific polymorphisms, which serve as targets for alloreactive T lymphocytes. However, it is also possible that some of the host's normal T cells are able to detect discrete antigens on tumor cells. Cytotoxic T lymphocytes (CTLs) recognize processed intracellular proteins presented as short peptide fragments together with MHC molecules on the cell surface. Hence, internal proteins unique to the malignant clone may act as tumor-specific antigens for CTLs. Several human malignancies contain novel proteins, such as mutated oncogenes or fusion proteins generated by chromosomal translocations.[79,80] Even normal proteins can elicit CTL responses if they are expressed in higher-than-usual quantities; tyrosinase and the MAGE series of proteins in melanoma cells are two good examples.[81] If tumor cells are able to process and present these tumor-specific peptides, then it is possible that a malignancy-specific response could be generated in the absence of any other host reactivity. Exploration of this possibility and of the effector mechanisms involved is easiest when the target antigens have been identified and characterized. Once this has been done, gene transfer affords an attractive mechanism for enhancing or amplifying particular effector functions.

Epstein-Barr virus (EBV) infection provides an excellent model system to examine genetic enhancement of immune effector function. EBV is a latent herpesvirus that infects more than 90% of the population. Primary EBV infection is usually a self-limited process followed by a life-long latency in oral epithelial cells and in B cells. The virus is also associated with a range of malignant diseases, not only of B and epithelial cells, but also of T cells, NK cells, and muscle. [82] All EBV-positive tumors reflect the latent life cycle of the virus. That is, with any given type of EBV-related malignancy, only a small proportion of the viral genome is expressed, and the differential regulation of these genes is one of many strategies the virus uses to evade the immune response.

### Lymphoproliferative Disease

Latent EBV infection can become active and induce lymphoproliferative disease (LPD) in children who have received solid organ or allogeneic stem cell transplants or who are receiving immunosuppressive therapy, or who lack an efficient CTL response for any other reason. The reported incidence of EBV-LPD among persons undergoing solid organ transplantation ranges from 1% to 10%, with the highest risk in seronegative recipients and patients receiving higher doses of immunosuppressive agents. Risk factors include *in vitro* T-cell depletion of donor marrow, use of a mismatched family donor or closely matched unrelated donor, and intensive immunosuppression.[5]

The onset of EBV-LPD is usually preceded by a large increase in viral load in recipients of either solid organ [83,84] or stem cell transplants.[85,86] The majority of these cases are B cell lymphomas or histologically high-grade non-Hodgkin's lymphoma of the immunoblastic or undifferentiated large cell type, [87] which respond poorly to cytotoxic therapy. The malignant cells express latent cycle virus-encoded antigens (EBNA1, 2, 3A, 3B, and 3C and LMP1, 2a, and 2b), most of which are targets for virus-specific immune activity.[88] The immunogenicity of these antigens has been exploited to treat posttransplantation EBV-LPD with unmanipulated donor T cells. Sloan-Kettering investigators reported responses to donor T cells in 20 of 22 patients undergoing stem cell transplantation. [89] Others have also had success with this strategy but have shown lower response rates and a significant risk of GVHD.[90] EBV-specific CTLs can be used as either prophylaxis or treatment for this disease. By genetically marking these cells using retroviral vectors, it is possible to track their behavior and survival *in vivo*.

***Prophylaxis with Cytotoxic T Lymphocytes*** Thirty-nine patients were treated with infusions of CTLs after transplantation of T-cell–depleted stem cells. None developed LPD, compared with 11.5% of a comparable untreated historical control group.[91,92] and [93] Gene-marked EBV-specific CTLs persisted in the patients for as long as 60 months, reconstituted immune responses to EBV, and reduced the high virus load seen in approximately 15% of patients. A study from Sweden recently confirmed the efficacy of EBV-specific CTLs in reducing the viral load in patients with high EBV-DNA levels posttransplantation. [86]

***Treatment with Cytotoxic T Lymphocytes*** Immunotherapy with antigen-specific CTLs can also be used to treat patients who develop overt lymphoma. In one study, two of three patients with this complication responded well to CTL infusion. One had a biopsy-proven accumulation of gene-marked CTLs at the disease site, [93] but there was a marked inflammatory reaction during the therapeutic response, underscoring the benefits of a prophylactic approach. The third patient did not respond and died of progressive disease 24 days after CTL therapy. This failure was traced to a deletion in the EBNA3B gene in tumor cells that removed immunodominant

epitopes, thereby inducing resistance to killing by CTLs.[94] Such escape mutants can compromise results, even when polyclonal CTL lines are used, and present a particular problem in patients with a large tumor load. Because production of EBV-specific CTL may be laborious, efforts have been made to use nonspecific T cells that are transduced with the thymidine kinase gene, allowing their destruction by ganciclovir should they produce adverse effects such as GVHD. Bonini et al.[95] used this approach to treat patients with posttransplant leukemic relapse or EBV-associated lymphoma. The transduced cells provoked GVHD in three patients, which resolved in two after treatment with ganciclovir. Several other patients developed immune responses to the transgenes, which may have reduced the longevity of the transferred cells, limiting their benefits.

***Hodgkin's Disease*** In North America, 50% of Hodgkin's tumors are EBV positive, expressing limited viral antigens in a type 2 latency pattern. In one study, EBV-specific CTLs were prepared from eight patients with advanced relapsed disease, gene marked, and reinfused as antitumor therapy.[96] Follow-up analyses showed *in vivo* expansion and long-term persistence of CTLs specific for LMP2, one of the Hodgkin's-expressed EBV antigens. There was also a reduction of EBV load and evidence for penetration and selective accumulation and expansion of CTLs at tumor sites. Despite resolution of type B symptoms and stabilization of disease, none of the patients had a complete response and all subsequently progressed.[96] Gene transfer may have an important role to play in improving this outcome. In one strategy, based on the use of CTLs specific for the subdominant antigens expressed in this disease, dendritic cells are transduced with an adenoviral vector encoding a full-length LMP2 gene. The CTLs recovered after exposure to these gene-modified cells are highly biased toward recognition of the LMP2 antigen. Another approach centers on the mechanisms by which Hodgkin's cells evade immune surveillance. Such cells secrete a number of T helper cell 2 (Th2) cytokines and chemokines, such as thymus and activation-regulated chemokine (TARC) and IL-10, that can inhibit to CTL immune responses. TARC recruits Th2 cells, which secrete IL-4 and thus contribute to the pro-Th2 and anti-Th1 environment already induced by the secretion of IL-10. To overcome this inhibition, CTLs can be transduced with the CCR4 gene, which encodes the chemokine receptor for TARC. It is hoped that TARC-expressing, LMP2 specific CTLs will be recruited to tumor tissues.

***Chimeric T-Cell Receptors for the Targeting of Hematologic Malignancies***

Tumor-specific T lymphocytes can also be produced by genetically modifying human T cells to express tumor antigen-specific chimeric immune receptors ("T-bodies").[97] This tactic is based on the observation that engagement of a single T cell or Fc receptor chain suffices to induce cellular activation.[98] One can generate chimeric receptors by joining the heavy- and light-chain variable regions of a monoclonal antibody, expressed as a single-chain Fv molecule, to the TCR-z ( Fig. 17-1), or Fc-g immune receptor domain. To promote functional recognition of its target antigen, the single-chain Fv is disengaged from the plasma membrane, usually by insertion of an immunoglobulin hinge-like domain. Antigen stimulation of the extracellular component of the chimeric receptor results in tyrosine phosphorylation of immune-receptor activation motifs present in the cytoplasmic domain, initiating T-cell signaling to the nucleus. Human T lymphocytes genetically engineered to express the recombinant receptor genes were capable of specific lysis and cytokine secretion on exposure to tumor cells expressing the relevant target antigens.[98] Adoptively transferred chimeric receptor–transduced cells were protective in murine tumor models.[98]

T cells with chimeric receptors have numerous advantages over immunotherapies based on monoclonal antibodies or T lymphocytes alone. They can be directed toward any native tumor- or viral-associated antigen for which a monoclonal antibody exists, making this strategy applicable to a wide variety of malignancies and viral diseases. In contrast to the lengthy process of CTL selection by standard methods, characterization and expansion of lymphocytes with native specificity for target antigens allows one to generate large populations of antigen-redirected T lymphocytes in a matter of weeks. Because chimeric T-cell receptors provide T-cell activation in a non–MHC-restricted manner, they permit cells to evade the major mechanisms by which tumors avoid T-cell recognition, such as downregulation of HLA class I molecules and defects in antigen processing. The absence of MHC restriction may also allow a "universal" cytotoxic T cell to be generated, although immune recognition of such foreign cells may reduce their clinical value. T-body–mediated effector functions are more likely to eradicate tumor cells than humoral immune responses alone, in that cytokine secretion upon T-cell activation recruits additional components of the immune system, amplifying the antitumor viral immune response. Finally, unlike intact antibodies, T cells can migrate through microvascular walls and penetrate the cores of solid tumors to exert their cytolytic activity.

Ensuring long-term persistence of transferred T cells in the host is a major challenge in adoptive immunotherapy. Rapid clearance of modified T-effector cells may be partly overcome by humanization of currently available hybridoma antibodies or by the generation of fully human single-chain antibodies by phage display technology. T lymphocytes may be locally lysed by factors secreted by tumor cells, and the surviving modified T lymphocytes may lose their antitumor viral reactivity by returning to a resting state. Although T-cell activation was not a prerequisite for efficient signaling through the chimeric receptor *in vitro*,[99] the functional capabilities of T cells with chimeric receptors in situations in which costimulation is limiting remain to be determined. To avoid the functional inactivation of T lymphocytes expressing chimeric receptors *in vivo*, it may be necessary in future designs to engage relevant co-receptor molecules.

The specific properties of the antibody used for antigen recognition may also be crucial for effective receptor stimulation. Signaling through native TCRs requires low-affinity binding to allow for serial triggering of a large number of these receptors,[61,100] yet the majority of antitumor antibodies used in the clinic bind with high affinity. Whether such binding is optimal for the efficient lysis of tumor cells by T bodies remains to be determined. Similarly, the target antigen for chimeric receptor–mediated immunotherapy should be carefully chosen, as receptor binding by shed tumor antigens or downregulation of the target antigen represents potential mechanisms of immune system evasion by tumor cells. Ideally, the targets of chimeric receptors constitute part of the tumor cell phenotyping.

Thus far, chimeric receptors have been developed for a variety of antigens associated with solid tumors, including neu/HER2,[101] folate-binding protein, carcinoembryonic antigen,[102] tumor-associated glycoprotein-72,[103] renal tumor-associated antigen, CD30,[104] and CD33.[105] For pediatric malignancies, studies are planned for patients with neuroblastoma and Ewing's sarcoma.

**Modification of Host Cytotoxic Drug Sensitivity**

An increased understanding of cytotoxic drug resistance has suggested gene therapy approaches to protect normal host tissues from the toxicity of chemotherapy. If, for example, hemopoietic stem cells could be rendered resistant to one or more cytotoxic drugs, it might enable them to resist the myelosuppressive effects of cytotoxic drugs during cancer therapy, allowing longer or more intensive therapy that could cure additional patients.[106]

The *MDRI* gene has been the most widely considered for human therapy. Its product, P-glycoprotein, functions as a drug efflux pump and confers resistance to many chemotherapeutic agents.[107] The feasibility of using *MDRI* to protect hemopoietic cells has been demonstrated by murine experiments, and retroviral transfer of *MDRI* to murine clonogenic progenitors conferred drug resistance both *in vitro* and *in vivo*.[108] These experiments with MDR1-containing vectors prove the principle that drug resistance genes can be used to attenuate drug-induced myelosuppression. It is likely that other drug resistance genes could function analogously. DNA-methylguanine methyltransferases (MGMTs) are enzymes that repair DNA damage induced by the nitrosoureas, a class of alkylating agents used widely in cancer chemotherapy. Preliminary data suggest that retrovirally mediated gene transfer of the human *MGMT* gene to mouse bone marrow cells results in protection of murine progenitors from toxicity produced by carmustine.[109] Other drug resistance genes, including those for dihydrofolate reductase[110] and topoisomerase 11, are also under consideration for clinical testing.

The clinical application of drug-resistance gene transfer has several potential pitfalls. The low efficiency of stem cell transduction and poor gene expression observed in the earliest clinical protocols resulted in no selection of gene-modified cells and hence no *in vivo* protection.[111,112] Improved transduction technologies using fibronectin and altered combinations of growth factors are beginning to result in *in vivo* selection, albeit not yet to clinically useful levels.[113] There remains the risk of transferring the genes to neoplastic cells that contaminate the hematopoietic stem cell (HSC) graft and produce drug-resistant relapse. Finally, toxicity to nonprotected organs, including gut, heart, and lungs, may rapidly supervene when marrow resistance allows intensification of cytotoxic drug dosages. The approach will only come to full fruition once it becomes possible to target normal tissues *in vivo* and transduce them with high efficiency.

**Gene Marking of Hemopoietic Progenitor Cells**

Not all of the applications of gene transfer to patients with malignant disease are directly therapeutic in intent. Gene marking of hemopoietic cells provides no immediate benefit to patients, but the information from these studies can be used to improve therapies that incorporate autologous HSC transplantation as a means of eradicating hematologic malignancies.[114]

Autologous HSC rescue has shown promise as effective treatment for leukemias and lymphomas (and perhaps for some solid tumors)[115,116,117,118] and[119] although disease recurrence continues to be the major cause of treatment failure. When the tumor originates from or involves the marrow, relapse could originate from malignant cells persisting in the patient, in the rescuing HSCs, or in both. Concern that the HSCs may contain residual malignant cells has led to extensive evaluation of techniques for purging these cells.[116,120,121] However, no method has been unequivocally shown to reduce the risk of relapse in naturally occurring disease, and

purging techniques usually slow engraftment due to damage to normal progenitor cells.

In the gene marking studies conducted to date, gene transfer has been used to address biologic questions about clinical issues related to bone marrow transplantation (BMT). More specifically, gene transfer has been used after autologous BMT to determine the source of relapse and to learn more about the biology of normal marrow reconstitution and how best to accelerate this process.

### *Source of Relapse after Autologous Bone Marrow Transplantation*

Although autologous BMT appears to improve survival in many pediatric malignancies, it does not eliminate relapse as the major cause of treatment failure. The possibility that reinfused malignant cells may contribute to disease recurrence has led to extensive evaluation of techniques for purging marrow to eliminate residual malignant cells, although it has been unclear whether such precautions are necessary. In one study, attempts were made to resolve this issue by marking the marrow at the time of harvest with a retroviral vector, and then determining if the marker gene was present in malignant cells at the time of relapse. [2] Among 12 patients with acute myeloid leukemia who were studied in this manner, four relapsed, two with cells that contained the marker gene. [2] Similar results have been obtained in patients with neuroblastoma and with chronic myelogenous leukemia.[122] These data show definitively that marrow harvested from patients in apparent clinical remission may contain residual tumorigenic cells and that these cells can contribute to disease recurrence. The implication is that effective purging will be one requirement for improving the outcome of autologous BMT.

### *Gene Transfer to Normal Cells*

The efficiency of gene transfer to normal marrow progenitor cells can also be determined from marker studies. By phenotypic (neomycin resistance) and genotypic (polymerase chain reaction amplification), analysis, the *neo* gene was present in approximately 15% of hematopoietic progenitor cells after autologous BMT in children.[3] Somewhat lower levels were found in adults.[44,123] The marker gene continued to be detected and expressed for up to 7 years in the mature progeny of marrow precursor cells, including peripheral blood T and B cells and neutrophils. These results suggest that true stem cells, and not simply lineage-committed progenitors, were transduced by this method.[3] Overall, the results are encouraging, as the levels of gene transfer in children were higher than one would predict from animal models. This gain can be attributed to the fact that marrow was harvested during its regeneration after intensive chemotherapy, when a higher-than-normal proportion of stem cells are in cycle. Second-generation studies of marrow marking have begun. In one effort, two distinguishable gene markers in two related retroviral vectors are being used to ascertain whether marrow purging is superior to no purging, as well as the relative efficacy of two different purging techniques within a single patient.[124]

### Ex Vivo *Expansion Studies*

Double gene marking is also being used to determine directly which *ex vivo* or *in vivo* combination of cytokines will increase the proportion of long-lived marrow-repopulating cells that enter the cell cycle, thereby reducing the period of marrow hypoplasia and immunodeficiency that follows autologous stem cell transplantation. In humans, it certainly appears possible to use growth factors to increase by ten- to 50-fold the numbers of hematopoietic progenitor cells, as well as the efficiency of gene transfer to levels as high as 50%. However, such *ex vivo* data are not always reflected by results *in vivo*. In primate and human studies, transplantation of marrow treated *ex vivo* with growth factor combinations that greatly augment both progenitor numbers and gene transfer rates can yield disconcertingly low levels of long-term gene expression *in vivo*.[44] The likeliest explanation for this seeming paradox is that many of the growth factors intended only to induce cycling in marrow stem cells also induce their differentiation and the loss of their self-renewal capacity. More recent efforts, using modified growth factors combined with fibronectin or the recombinant fibronectin fragment retronectin have resulted in greatly improved levels of gene transfer and detection *in vivo*.[113,125]

It is possible to use the marker gene technique to evaluate whether any increase in progenitor cell numbers and transducibility produced by growth factor combinations and cell culture devices *ex vivo* has an effect *in vivo*. Once again, the use of two distinguishable vectors to mark each patient's marrow allows comparison of treatment regimens within a single patient, so that very small patient samples can be used to answer difficult study questions. This technique is also being used to compare peripheral blood- and marrow-derived progenitor cells for their relative ability to reconstitute hematopoiesis after BMT or other marrow ablative treatments.

### Safety of Gene Transfer in Pediatric Malignancy

Because most studies of pediatric malignancy have involved *ex vivo* gene transfer, concerns about direct vector-mediated toxicity are not as acute as in protocols in which vast numbers of vector particles are administered parenterally. To date, more than 200 patients have received genetically modified cells in clinical trials in pediatric disease, including more than 60 who have received genetically modified hemopoietic progenitor cells. With a maximum follow-up of 9 years, and a total patient follow-up of more than 250 patient years, no adverse events attributable to the gene transfer process have been identified. In particular, there has been no evidence of lymphoproliferative disorders. Of potential concern, however, are reports showing a cellular immune response directed against transferred gene products.[126] Clearly, if an immune response is regularly generated against the product of any transferred gene, the value of gene therapy would greatly diminish. Prolonged follow-up of patients receiving genetically modified cells is essential at this early stage of technology development.

## CONCLUSION

We have far to go before the extraordinary potential of gene transfer for therapy of pediatric malignancies can be fully exploited. However, it is important to remember that most advances in medicine proceed incrementally, and that gene transfer is already being used successfully to complement conventional therapies for malignant hematologic disorders. The benefits of this new technology can only increase as current limitations are progressively—albeit slowly—surmounted.

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# 18

# CANCER CLINICAL TRIALS: DESIGN, CONDUCT, ANALYSIS, AND REPORTING

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## INTRODUCTION

The major concern of pediatric oncologists is the cure of children with malignant diseases. The past four decades have witnessed a remarkable improvement in the life expectancy of children diagnosed with cancer, the cumulative result of many modest advances. Demonstration of the benefit of these advances relies on the systematic use of therapeutic clinical trials.

A clinical trial is an experiment that attempts to answer a medical question, most often about the effect of a therapeutic intervention on the outcome of a disease. The experiment is carried out under conditions determined in advance by the investigator, who uses a specified methodology to test a hypothesis. A valuable experiment is one that addresses a nontrivial question in a way that provides unambiguous, reliable, and easily interpreted results. This chapter discusses the requirements of a valuable clinical trial in terms of design, analysis, and subsequent reporting, with attention to the particular problems in applying clinical trial methodology to the study of pediatric cancer. Our intention is to provide information useful in mounting clinical trials and in reviewing the published efforts of other investigators.

The rationale for conducting clinical trials in pediatric oncology goes beyond the demonstrated utility of the therapeutic approach. It is based on the need to reach accurate conclusions about the benefit of toxic and expensive therapies. Nonexperimental research, in which reasoning rather than observation is used to reach conclusions, or flawed experimental research, in which the therapeutic intervention is inconsistent or uncontrolled, can severely restrict progress toward the cure of childhood cancer by erroneously supporting the value of what is actually useless or harmful treatment.

Cancer clinical trials are conventionally categorized into three types, derived from usage adopted by the National Cancer Institute (NCI) drug development program. [1] A phase I trial investigates the toxicities associated with a particular agent and determines the maximum tolerated dose (MTD) or the appropriate dose with a given schedule and route of administration for use during subsequent efficacy studies. [2] A phase II trial estimates the activity of the agent against individual tumor types. [3] A phase III trial assesses the activity of the agent in a comparative fashion, with reference to standard therapy or, in some cases, the natural history of the disease. [4] The application of these concepts now extends beyond new drug development to include the evaluation of any new therapeutic approach, but the progression (from assessment of toxicity through estimation of efficacy and establishment of superiority, equivalence, or inferiority through direct comparison) remains constant. When clinical trials are used to evaluate a treatment regimen rather than an individual agent, phases I and II are sometimes combined. These trials are referred to as *pilot studies*.[5]

## PLANNING A CLINICAL TRIAL

The process of planning a clinical trial culminates in the generation of a protocol, which is a written guide to the experiment to be conducted. As a procedural guide, the protocol helps to ensure that the investigation is carried out uniformly. An equally vital role is to encourage investigators to develop a precise, well-justified, and practical approach to the study. Adherence to the organizational requirements of a well-constructed protocol should guide the investigator through the planning process, so the resulting clinical trial is significant, feasible, and likely to provide a definitive answer to an important question.

An essential step in the design of the clinical trial is the approach to regulatory affairs. In the narrowest sense, this means attention to documentation of compliance with study requirements such as eligibility criteria, drug administration, laboratory and clinical follow-up, and response and toxicity measurement for each individual subject entered onto the trial. In a broader sense, regulatory affairs encompass the sponsors' and investigators' obligations to ensure that the conduct of the study falls within legal and ethical guidelines set forth by the relevant agencies [e.g., in the Good Clinical Practices Guidance document adopted by the U.S. Food and Drug Administration (FDA) as well as regulatory agencies in other countries]. [6] Although some regulatory activities, such as obtaining informed consent from subjects, do not take place until the clinical trial is activated, careful consideration of the requirements starting early in the trial design process may circumvent many difficulties and prevent frustrating delays in the initiation of the study.

The sponsor of a clinical trial may be the investigator initiating the trial, a pharmaceutical company, or an entity such as the NCI's Division of Cancer Treatment and Diagnosis. The sponsor is legally responsible for the overall conduct of the trial. Early in the design of the study, the sponsor must consider whether there are special regulatory requirements for the particular type of research in question. For example, if the trial involves an investigational agent, the sponsor must submit to the FDA an Investigational New Drug application. The Investigational New Drug application details the preclinical pharmacology and toxicology experience, the relevant manufacturing information, and details of the proposed clinical trial. The sponsor must allow 30 days after the Investigational New Drug application is submitted for the FDA to complete its safety review before initiating the clinical trial.

The sponsor of a protocol involving an investigational agent is also responsible for ensuring that individual investigators and participating institutions fulfill their regulatory responsibilities. All participating investigators must complete FDA Form 1572 documenting their qualifications as well as their agreement to conduct the study according to federal regulations. In addition, at each institution the investigators must obtain appropriate review of the protocol by an Institutional Review Board (IRB). The IRB's duty is to ensure that human subjects participating in clinical trials are protected from research-related risks. The philosophy behind such protection is stated in the Nuremberg Code, the Declaration of Helsinki, and the Belmont Report, and codified in the United States in federal law. [7,8,9,10,11] and [12] Central to the protection of human subjects is the concept of informed consent, which is discussed in detail in Chapter 48. In addition, the potential risks and benefits of the research

must be shared fairly among the general population (justice), the risks to the individual must be minimized and the benefits maximized (beneficence), and the rights of the individual to decide whether to assume research risks must be observed (respect for persons). Furthermore, because children are considered a highly vulnerable population, they are afforded additional protections, such as a requirement in most instances that they stand to have individual benefit from participating in research that exposes them to more than minimal risks. It is important to note that good scientific design of the clinical trial is an absolute requirement for protection of human subjects, not only because potential benefits will be maximized and risks minimized in a good trial but also because a poorly designed study that is unable to answer the research question cannot offer patients or society any benefit to offset the potential risk of participation.

Once the study is open, the sponsor and investigators are responsible for strict adherence to the principles of good study conduct. Both individual patient data, submitted on case report forms developed during the protocol design process, and periodic study summaries that review the overall status of the protocol are required. At a minimum, yearly review must be conducted by the IRBs at each participating institution. The sponsor must ensure that there has been compliance with the protocol requirements, that the data reported are accurate, and that all study procedures have been followed. For every subject entered on the trial, there must be documentation that diagnostic and eligibility criteria were met, study treatment was correctly administered, required observations were performed, appropriate endpoints were documented, adverse events were appropriately reported, and so forth. In particular, adverse event reporting to the appropriate regulatory bodies is an important part of every investigator's obligations, and the procedures for such reporting should be carefully outlined in the study protocol. The accuracy of data submitted on the case report forms for each subject is usually verified using audits of the medical records of some or all participating patients. Finally, compliance with study procedures ensures that appropriate IRB approvals, individual subjects' informed consent, and study drug accountability are all correctly documented. [13,14] Unavoidably, a certain tension exists between a clinical investigator's role as caregiver and as scientist, and between the study participant's role as patient and as research subject. Similarly, there is tension within our society between the desire to further scientific and medical understanding for the good of all, and the need to protect each individual's safety and autonomy above all else. Although it is unlikely that these tensions can be completely resolved, they can be minimized by scrupulous attention to regulatory requirements that are designed to promote responsible clinical research while protecting individual subjects from undue risk.

The components of a typical protocol are listed in Table 18-1. The eventual success of any clinical trial depends heavily on the care and skill devoted to the consideration of each of these items. Given the substantial time, effort, and expense required of collaborators and patients during the trial and the potential damage associated with an incorrect result, the responsibility of critical protocol development cannot be overemphasized. Proper attention to these requirements helps to maximize the value of the experiment and its probability of impacting the medical community.

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| Objectives |
| Background |
| Patient eligibility criteria |
| Design |
| Treatment plan |
| Drug information |
| Criteria for evaluating treatment effect |
| Clinical and laboratory data to be accessioned |
| Statistical considerations |
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**TABLE 18-1. TYPICAL PROTOCOL SECTIONS**

### Objectives

The first protocol requirement is a statement of objectives. This statement should be limited to the actual research question, such as, “in stage I Wilms' tumor, to determine whether 6 months of postoperative treatment with vincristine and dactinomycin (actinomycin D) results in event-free survival inferior to that observed with 15 months of vincristine and dactinomycin.” Less exact versions, such as, “to reduce the vicissitudes of treatment of Wilms' tumor,” should be avoided. The inability to set down a sharply defined objective is indicative of a poorly developed research plan, as is commonly seen when a patient management guide masquerades as a research protocol. A precise statement of the objective focuses the investigator on the conditions required to conduct the chosen experiment and allows him or her to assess the feasibility of the trial in terms of the available resources.

Of particular importance in pediatric clinical trials is the limiting of the number of study objectives, because the available patient population is not large enough to provide reliable answers to many experimental questions. The objectives should reflect the most important hypotheses that require evaluation, although this goal is not always possible to achieve. The restrictions imposed by limited numbers of patients often necessitate multi-institutional collaborations. Although such arrangements do increase patient availability, they often cause difficulty in arriving at a common research plan because of the multiplicity of opinions.

Investigators should select objectives that can provide useful information regardless of whether the study results are positive or negative. This end is possible when a biologic hypothesis is tested in a carefully controlled manner. [15]

### Background

The background section provides the underpinnings for the entire protocol by presenting the arguments for conducting the clinical trial and for selecting the specific experimental conditions enumerated in subsequent sections. As a crystallization of the thoughts that led to the proposal's existence, the background section should provide adequate justification for mounting what will be a difficult, time-consuming, and costly exercise. This section offers a review of the pertinent medical literature, presented in such a way as to convince the reader that the question is compelling, has not previously been answered, and can feasibly be addressed with existing resources. The plausibility of the hypothesis must be supported through documentation of its biologic or experimental basis. Information on prognostic factors pertinent to patient selection should be reviewed, and material relevant to design considerations should be included. Discussion of the significance of the proposed trial should be within the perspective provided by previous clinical trials. Background information for phase I protocols should include preclinical ( *in vitro* and animal) data and results of human trials with the experimental agent. The rationale for using the agent in children should be presented with justifications for the chosen schedule and starting dose.

Phase II protocols additionally should include a summary of available data on toxicity in adults and children and a justification of the choice of malignant diseases against which the agent will be assessed. Phase III protocols require extensive background sections that establish the basis and significance of the protocol within the framework of previously ascertained results.

### Patient Eligibility Criteria

The protocol should define the characteristics of the patient population to be studied, including factors such as diagnosis, extent of disease (stage), age restrictions, allowable prior therapy, physiologic and performance status, and any other conditions the investigator wishes to specify, such as the expression of particular biologic markers by malignant cells. In the United States, informed consent must be obtained from patients or parents (depending on patient age) to establish eligibility (see Chapter 48). The population defined by these criteria is the one to which the study results apply; thus, patient criteria must be carefully chosen and precisely defined.

Although the investigator may wish to restrict study entry to a homogeneous patient population, the realities of biologic variability preclude this possibility; until all factors affecting prognosis are elucidated, study populations will consist of mixtures of patient groups, each of which may behave differently under the conditions of the experiment. The relatively small number of pediatric patients available for clinical trials presents further problems, because overly restrictive criteria can yield insufficient entries to answer the study question. Frequently, determining the optimal balance is difficult.

Homogeneity of patients is more important in phase I and phase II studies, in which information about biologic activity is sought. Phase I protocols generally specify patients in whom conventional therapy has failed yet who have sufficiently intact organ function for accurate assessment of toxicity. Enough time (usually 2 to 4 weeks) should have elapsed since the most recent antitumor therapy to ensure that the short-term effects of that treatment have subsided. Patients with solid tumors

should not have bone marrow dysfunction, so toxic effects on normally functioning marrow can be assessed. This criterion does not apply to patients with leukemia. Patients with any histologic type of cancer are usually eligible for phase I studies, although patients with solid tumors and leukemia must be evaluated separately to determine the MTD.[2] Patients with leukemia commonly tolerate higher doses of myelosuppressive agents; moreover, marrow suppression is sought in leukemia patients but is avoided in patients with solid tumors.[4,16] Regardless of tumor type, life expectancy sufficient to permit assessment of drug-related effects should be specified.

Phase II studies are ideally conducted with previously untreated patients to avoid the problems of acquired drug resistance and diminished tolerance associated with prior treatment. Because most childhood cancers are potentially curable at diagnosis with conventional therapies, however, phase II studies in children generally specify that standard therapy must have failed in these patients. Phase II evaluation has been used increasingly before definitive therapy [17] (“experimental window,” “upfront phase II window”) for certain clinical situations, such as metastatic osteosarcoma, [18] unresectable rhabdomyosarcoma,[19] or disseminated neuroblastoma in children older than 1 year[20] in whom the outcome of conventional therapy has been unsatisfactory. Although the value of this approach in establishing reliable estimates of activity in high-risk situations is promising, [18] a clear concern is that delay in starting definitive therapy may compromise patient outcome if the agent used in the upfront window is inactive.[21,22] and [23] Proponents of the method counter that responses during phase III therapy in patients randomized to receive initial phase II therapy with an agent subsequently found to be inactive are similar to those seen in patients randomized to phase III therapy only.[20,24] Nevertheless, as the data cited by proponents are not definitive, consideration should be given to the possibility that patient outcome can be compromised if agents or combinations of agents are used in the upfront window in the absence of strong preclinical or clinical rationale for such evaluation. A meeting of clinicians, clinical researchers, patient advocates, and bioethicists in 1997 discussed these issues. Participants affirmed the need to ensure that patients and their parents are fully informed about the role and optional nature of upfront window research in the patient's overall treatment plan.[25]

Phase II protocols additionally specify histologic diagnoses acceptable for entry because the end point often depends on tumor type. Patients are also generally required to have measurable disease, so antitumor response can be reliably assessed.

In phase III studies, the issue of generalizability of results obtained in the study population to the affected population as a whole becomes more important. Despite (or perhaps because of) the rarity of cancer in children, NCI-supported pediatric clinical trials cooperative groups annually accrue more than one-half the eligible patient population, including those in racial and ethnic minorities. Because this large patient sample is representative of the affected population, the fundamental principle is to select patients with reasonable potential to show benefit, whether due to decreased toxicity or improved survival, from the experimental therapy. Patients whose prognosis is so good that therapy is unlikely to have an observable positive effect during the course of the study should be excluded. It is also appropriate to exclude patients with contraindications to any of the study treatments or those who have other serious conditions that may interfere with administration of therapy.

## Design

The design section of the protocol provides a brief overview of the study's structure and describes the methods by which the objectives are to be met. The minimum requirements for this section include the plans for patient allocation and the criteria by which the experimental effect is to be evaluated (i.e., study end points). In our previous example of a phase III clinical trial in stage I Wilms' tumor, in which the goal is to evaluate event-free survival, the design would specify that after surgical removal of the tumor and 6 months of adjuvant chemotherapy with vincristine and dactinomycin, patients would be randomly allocated to no further therapy or to an additional 9 months of chemotherapy, with tabulation of event-free survival times on both regimens.

### *Phase I Trials: Specific Designs*

A consensus guideline on the conduct of pediatric phase I trials has recently been published.[26] Phase I studies using children typically use a standardized design in which cohorts of three patients are treated with identical doses of the experimental agent and are then observed for acute toxicity. If none of the three patients experiences dose-limiting toxicity (DLT), the dose is escalated. At any dose level, when DLT is observed in one patient, the cohort is expanded to six patients. The MTD is defined as the dose level immediately below that at which two patients (in a cohort of three to six) experience DLT, and is usually also the recommended starting dose for phase II trials. If myelosuppression is the DLT, consideration is often given to enrolling patients with limited prior therapy onto the study, because an inappropriately low MTD may be defined if only heavily pretreated patients, who may tolerate therapy less well, are enrolled in the phase I trial.[27] Similarly, separate phase I trials of the same agent are usually conducted in patients with leukemia and with solid tumors, because more prolonged myelosuppression is acceptable in the treatment of hematologic compared with nonhematologic malignancies.

The starting dose for adult phase I trials is based on animal toxicology studies and generally is one-tenth the dose lethal to 10% of a cohort of mice, expressed in mg per $m^2$ (0.1 MELD10).[28] Stepwise dose increases are specified in the protocol. Often, a modified Fibonacci scheme is used to determine the escalations for successive cohorts.[29] The starting dose is increased by 100% in the first escalation, and subsequent doses are increased by adding 67%, 50%, 40%, and 33% of the dose established by the preceding cohort. The diminution of the escalations reflects increasing caution as one becomes farther removed from the starting dose. An alternate version of this scheme is to double the dose until “biologic activity,” such as mild myelosuppression, is observed, then to institute the diminishing escalations of the Fibonacci series.[30]

If data from phase I trials in adults are available, an efficient method is to start children's trials at 80% of the adult phase II dose, or at 80% of the dose at which biologic activity was observed in adults, bypassing levels that are presumably safe in children but are unlikely to be of benefit. In the absence of DLT, dose escalation should continue in children beyond the phase II dose established for adults, because children often display greater tolerance to chemotherapy, and the goal is to deliver the highest safe dose of a new agent in efficacy trials.[31] Starting doses derived from the adult MTD are presumably close to the childhood MTD, and escalation should proceed cautiously, using 30% increases over the preceding dose level.[31]

An alternative method for selecting the entry dose level and rate of escalation in phase I trials has been proposed by Collins and colleagues,[29,32] who advocate pharmacologic guidance based on preclinical toxicology in place of the empiric Fibonacci system. Although this method has been used by some investigators in phase I studies of new agents in adult patients, the value of this procedure in efficiently determining safe phase II doses for children remains to be determined.[33,34]

O'Quigley and colleagues[35,36] and [37] proposed a new approach to phase I studies in cancer aimed at moving to the MTD more quickly and thereby increasing both the efficiency of this early phase of development and the likelihood that patients treated in phase I will receive potentially beneficial treatment. This approach, the continual reassessment method, targets the dose to an “acceptable” toxicity level selected by the investigators. Bayesian methods are used to continually update the expected probability of toxicity based on the experience observed up to that point in the study. The continual reassessment method has been criticized on the grounds that it may lead too frequently to treatment at unacceptably toxic levels and may in many cases lead to longer phase I trials.[38,39] Modifications of the method that address these difficulties have been proposed.[39,40]

The current explosion in information about the molecular mechanisms underlying many malignancies is likely to result in an ever-increasing number of specifically targeted agents being brought into the clinic. Because most pediatric tumors are biologically distinct from adult tumors, some agents will be designed only for the treatment of pediatric cancers so that phase I trials of those agents in children must be conducted without prior information from adult studies. Furthermore, the appropriate phase I end point for such agents may be the determination of the dose that produces the optimal desired host response (the optimal biologic dose), rather than the MTD, for use in phase II studies of the agent.[41] Thus, the requirement for innovative strategies in pediatric phase I trial design is likely to increase as biologically targeted therapy becomes more common.

### *Phase II Trials: Specific Designs*

The conventional objective of phase II trials is to determine whether a new agent or treatment strategy appears sufficiently active to warrant further study. Activity is usually defined by objective tumor responses, although laboratory end points, such as immune parameters and cytogenetic normalization, are of increasing interest because of the advances in understanding of the malignant process. It is generally desirable to limit phase II studies to small numbers of patients to permit testing of the maximum number of agents or programs that require evaluation. Sample sizes for phase II studies have traditionally been set at 25 to 40 patients, but these numbers do not permit accurate estimation of tumor response rate. For example, if five responses are observed in 25 patients, the 90% confidence interval around the observed rate of 20% is [10%, 36%]. With 40 patients and eight responses, the 90% confidence interval around the same observed rate of 20% is [11%, 31%], narrower but still too wide to permit reliable distinction between uninteresting and moderate levels of activity. Thus, typical designs have been geared toward

identifying regimens whose response rates appear consistent with interesting levels of activity, rather than obtaining accurate estimates of response rates.

The simplest and perhaps most widely used phase II design was suggested by Gehan in 1961.[42] Gehan proposed a two-stage design, with the sample size in the first stage dependent on the minimum response rate of interest and the total sample size dependent on the expected variability of the estimate of the response rate (i.e., standard error). A sample size of 25 patients produces an estimate of response rate with a standard error no greater than 10%. The purpose of the two-stage design is to permit early termination if the activity level observed is clearly inconsistent with the minimum level of interest. For example, in a disease for which many agents are reasonably active, a new agent may be of interest only if it is associated with a true response rate of 25% or more. If this agent is given to 11 consecutive patients without a response, one would probably want to discontinue its use because a drug with a "true" 25% response rate would produce such a negative result less than 5% of the time. In a clinical situation for which few active agents have been identified, the threshold response rate of interest may be as low as 15% or even 10%. Table 18-2 shows the required first-stage sample size for different response rates. The total sample size should be at least 25, and larger samples are needed if small response rates are of interest.

| Minimal response rate of interest (%) | First-stage sample size[a] |
|---|---|
| 5 | 59 |
| 10 | 29 |
| 15 | 19 |
| 20 | 14 |
| 25 | 11 |
| 30 | 9 |
| 40 | 6 |
| 50 | 5 |

[a]If no responses are observed in these patients, the trial may be terminated with the conclusion that the true response rate is unlikely to be at or above the specified minimal level. If any responses are observed, additional patients are accrued.

**TABLE 18-2. FIRST-STAGE SAMPLE SIZES FOR GEHAN PHASE II TRIAL DESIGN**

Multistage phase II designs have been proposed as a means to improve the efficiency of phase II studies and to minimize the number of patients treated with inactive therapy. The Fleming design uses two or more stages and allows termination at any stage when the activity level appears too low or is sufficiently high to support moving to Phase III.[43] Chang and associates[44] and Simon[45] developed optimization strategies for the multistage Fleming-type designs in which sample size is minimized under specific conditions. Ensign and colleagues [46] provided a modification of Simon's approach for optimal designs by incorporating an initial stage after which the study would be terminated if no responses were observed, a feature of Gehan's original design for phase II studies. Zee and co-workers [47] developed a multi-stage procedure that considers early disease progression in addition to response as a primary end point and basis for decision making.

Other approaches to phase II trials have been described. Herson's design incorporates prior information about therapeutic efficacy into the decision rules. [48] Thall and Simon's[49] proposed Bayesian approach to phase II trials similarly incorporates prior information and continually updates the probability of response based on the accumulating observations. In this approach, sample size is not fixed; the trial continues until the probability of response is either high enough or low enough to warrant a decision regarding the desirability for further study of the agent or until a prespecified maximum study size is obtained. Sylvester and Staquet [50] and Sylvester[51] took a decision-theoretical approach, incorporating financial and ethical costs associated with making incorrect decisions about treatment efficacy and information available from earlier studies. Lee and co-workers [52] proposed a design that allows an inconclusive result as well as "positive" and "negative" outcomes.

If patient accrual is expected to be rapid and if multiple new agents are available for study, a randomized phase II design, as discussed by Simon and associates, [53] has some advantages over the sequential study of one treatment arm at a time. A randomized phase II study is simply the simultaneous implementation of two or more phase II studies with randomized assignment to the investigational agents. It does not permit formal statistical comparisons among the arms because sample sizes are too small for such comparisons to be done reliably. The randomization does ensure that no systematic bias is operating in the selection of study patients to receive each treatment, however. Thus, results from a randomized phase II study can be more reliably ordered in setting priorities for future studies than can results from sequentially conducted studies, in which patient selection patterns may have differed.

### *Phase III Trials*

#### Eligibility and Choice of Controls

Phase III trials compare the efficacy of an experimental therapy with that of a standard or control therapy. Because these trials are intended to determine definitively whether a new therapeutic strategy should be adopted, the design must be precise enough to avoid false-positive and false-negative results. In the study of pediatric cancer, phase III trials are usually feasible only in cooperative group or multicenter settings because of the relative scarcity of patients.

Investigators have debated issues relating to acceptable control groups for phase III studies. The optimal approach is a two-arm study, in which one group of patients receives standard therapy and the other receives experimental therapy. Alternatively, all patients can be treated with the experimental therapy, with results eventually compared with those achieved with a previous group of patients given what is currently considered standard therapy. This use of historical controls appeals to many physicians because it sharply reduces the number of patients required for study and because a nonrandomized study in which all patients receive the same therapy is easier to explain to potential subjects. These studies are inherently unreliable, however, because they have no protection against the possibility that different types of patients are treated with the two therapies.

As an example, suppose standard therapy for a particular category of patients is purely palliative, and a new, potentially curative therapy is being tried for these patients. The investigators elect to treat a series of patients with the new therapy and then compare the survival of these patients with that of a historical series treated with the palliative therapy. Several clear sources of potential bias exist in this situation. First, eligibility criteria are established for patients receiving the new therapy. Patients who appear close to death, who show evidence of organ dysfunction, who have received intensive prior therapy, or who refuse consent may not be treated with the new therapy. The historical series may not have been constructed with these (or any) eligibility criteria and thus may include some patients with an extremely poor prognosis who are not comparable with any patients treated with the new therapy. (Even if the same eligibility criteria are applied, changes in diagnostic techniques may affect patient selection.) Second, some patients considered for the new therapy meet the eligibility criteria but, for one reason or another, are not entered on the study. Perhaps these patients appear to be in worse condition than the required tests indicate; perhaps investigators are reluctant to enter infants, although these patients are technically eligible; perhaps the new therapy is expensive (e.g., bone marrow transplant), and only affluent patients are ultimately entered. Certain types of patients may then appear in different proportions in the historical and the experimental series. The comparison of survival or other outcome between these two series of patients is likely to be heavily biased if such patient characteristics are correlated with prognosis. Third, changes in supportive care procedures may improve survival apart from any fundamental population differences, and there is no way to adjust for this type of effect.

Thus, if differences in outcome are found [e.g., if the average survival with the experimental therapy is substantially longer than with the palliative (historical control) therapy] investigators will find it difficult to know how much of the difference to attribute to the new treatment and how much to attribute to fundamental variations in the patient populations or to changes in diagnostic techniques or supportive care. Although the statistical methods to "adjust" comparisons for some of these differences have been developed and are useful in many situations, one cannot measure and record all patient characteristics that affect prognosis. Many such characteristics undoubtedly remain to be identified. Thus, observed differences in outcome in historically controlled studies are always potentially attributable, to some extent, to causes that are not related to treatment and are always suspect. Such concerns are even more severe for comparisons made from databases in which patients were never treated on any protocol but received a regimen decided on by their physicians and themselves.

The most reliable way to generate unbiased comparisons between treatments is to allocate patients to treatment arms by randomization. A randomized trial is unquestionably the procedure of choice for the definitive evaluation of an experimental therapy. Large randomized studies do not always seem feasible, but they can sometimes be made feasible if investigators pool their efforts and develop a cooperative trial. Occasionally, even the multicenter approach is unsuitable. For example, relatively few cases of nonmetastatic medulloblastoma are diagnosed in toddlers each year, and a randomized trial using this patient population is probably

unrealistic. In these patients, a carefully done historically controlled trial, however imperfect, may be the only way to evaluate a new therapy.

### Sample Size

The required sample size for a randomized phase III study depends on (a) the minimum difference in outcome considered important to detect, (b) the levels of type I and type II errors considered acceptable, and (c) the expected outcome with standard therapy. Type I (a) error is the conclusion that the new treatment is better than the standard treatment when in fact it is not. The probability of a type I error is the significance level ( *p*) of the experiment and is denoted by a. Type II (b) error is the failure to conclude that the new treatment is superior to the standard when it actually is. The probability of a type II error is denoted by b; its complement (1-b) is called the *power* of the experiment.

The interpretation of the type I error depends on whether the test performed is “one-sided” or “two-sided.” A one-sided test considers differences in only one direction. For example, if treatments A and B are compared, a one-sided test will permit only two conclusions: A is better than B, or A is not better than B. A two-sided test permits a third conclusion: A is worse than B. Because a two-sided test allows for type I errors in both directions, a larger sample size is required to restrict the overall type I error to the desired level. Defining situations in which one-sided tests are appropriate is controversial. Some researchers maintain that such tests should not be used unless a difference in one direction is actually impossible; other researchers use one-sided tests whenever the expectation is clearly in one direction. Therefore, the investigators must specify *p* values as one-sided or two-sided, so readers can make their own interpretations of the degree of significance.

The specifics of the sample size calculation also depend on the type of end point of primary interest. A complete discussion of sample size determination for binary end points (e.g., yes or no variables, such as response) and extensive tabulations of sample size requirements according to the foregoing parameters were published by Fleiss.[54] Sample size considerations for time variables (e.g., survival time and remission duration) are discussed by George and Desu [55] and by Rubinstein and colleagues.[56] Both reports deal with the problem of “censored data”—survival times for patients who remain alive at the time of study reporting. These methods assume that the risk of an adverse event, such as death or relapse, is constant over time for any given patient, however. In many instances, particularly with pediatric tumors, a substantial cure rate can be anticipated, so the risk of recurrence should essentially disappear once a patient has been event-free for a certain period. Sposto and Sather developed methods for determining sample sizes in this situation. [57] Detailed expositions of general issues in sample size determination are given by Lachin,[58] Lachin and Foulkes,[59] Donner,[60] and Ellenberg.[61]

Conventionally, the type I error for testing results from phase III studies is set at 5%, and the type II error is set between 10% and 20%. This is done because incorrectly abandoning a standard treatment, with which investigators may have extensive experience, is considered a more serious error than failing to identify an experimental treatment that affords a moderate advantage. In general, the relative seriousness of the two types of error depends in part on characteristics of the treatments other than efficacy. A small a relative to b is appropriate if the experimental therapy is highly toxic, logistically difficult (e.g., requiring extensive hospitalization or multiple clinic visits), or very costly, whereas the standard therapy is relatively benign, simple, and inexpensive. In other circumstances, it may be more appropriate to set a and b more nearly equal. For example, if the new therapy is less intensive than the standard, the new therapy should be adopted only if it has not led to a worse outcome on average. In this case, one would worry as much if not more about failing to detect reduced efficacy than about falsely concluding that the standard, more intensive therapy was superior.

The size of the difference one is interested in detecting must be carefully considered, because the required sample size is extremely sensitive to this difference. For example, if one wanted to be 80% certain of observing a statistically significant ( $p < .05$) difference in event rates associated with two therapies when the true event rates were 20% and 40%, 91 patients per arm would be required. For event rates of 20% and 30%, however, the required sample size would be 313 per arm, and for rates of 20% and 25%, 1,134 per arm. Thus, detection of small differences, although possibly desirable, is not an achievable goal for most pediatric studies. On the other hand, the specified difference must be small enough so that a study resulting in “no statistically significant difference” is convincingly negative. If a study enters only enough patients to detect large differences reliably, smaller differences that may be clinically meaningful are unlikely to produce statistically significant results.

As an example, consider the hypothetical data in Table 18-3. The difference in complete response rates appears impressive: 50% versus 30%. The *p* value for this difference is .147, however, meaning that the probability of observing a difference at least this great if the two treatments were truly equivalent is 14.7%, which is suggestive but far from conventional significance. These data would be considered inconclusive. The observed difference is too large to conclude comfortably that the two drugs are equivalent, but the numbers are too small to exclude chance as the basis for the difference.

| | Treatment | |
|---|---|---|
| Complete response | Drug A | Drug B |
| Yes | 15 (50%) | 9 (30%) |
| No | 15 | 21 |
| Total patients | 30 | 30 |

**TABLE 18-3. HYPOTHETIC PHASE III TRIAL DATA**

## *Specialized Designs for Randomized Clinical Trials*

### Sequential Designs

A sequential design is one in which the total sample size is not fixed at the beginning but depends instead on the data accumulated as the trial progresses. Historically, the motivation for the use of sequential designs was the desire for efficiency, achieved by terminating an experiment as soon as the answer is “known.” In the context of clinical trials, the motivation is primarily ethical. If early results indicate that one treatment is producing substantially improved outcomes, it becomes difficult to justify the continued randomization of patients to the study. The application of simple monitoring plans, such as stopping the study as soon as the *p* value reaches .05, however, has been shown to lead to a far greater frequency of type I errors (false-positives) than the nominal .05 level would indicate. For example, McPherson[62] points out that if the data are reviewed ten times during the course of a study in which the true efficacies of the treatments are identical, the probability of observing a *p* value of .05 or less at least once is approximately 20%. Thus, the true type I error in this context is actually 0.20, not 0.05. The type I error increases with more frequent interim monitoring.

To deal with the problem of inflating type I errors by frequent evaluation of study results, several different approaches have been developed. All require the use of more stringent significance criteria than the usual 0.05 level. The most practical of these approaches are called *group sequential designs* because they are based on analysis of data at regular intervals, often semiannually, as groups of data are accumulated. The basis for these designs is that one must try to compensate for the extra opportunities to declare significance (and possibly make a type I error) by reducing the significance level of each test so, over the entire course of the trial, the probability of type I error remains at 5%. The most widely used design was proposed by O'Brien and Fleming. [63] Their method uses a sequence of *p* values for successive analyses: the first is exceptionally small, with the remainder gradually increasing so the final *p* value is close to the conventional level (typically .05). Other approaches are described by Pocock,[64,65] and Fleming and co-workers.[66]

Lan and DeMets[67] showed that the O'Brien-Fleming approach could be made more flexible by introducing a “use function” that specifies how rapidly the type I error may be used up, but it does not require prior specification of the total number of monitoring times or the intervals between these times. More recent work by these authors indicates that when the pattern of interim review changes as a direct result of the observed data, the overall type I error is affected, but the effect is small. [68] Jennison and Turnbull[69,70] and [71] developed a sequential design based on repeated confidence intervals. This approach is appealing because it provides more

information about the possible treatment effect than does a simple *p* value.

These designs allow for the possibility of early termination only if one treatment appears markedly superior to the other. It is frequently the case, however, that as the data accumulate, the experimental treatment appears no better (or perhaps even somewhat worse) than the standard. Just as an intuitive but nonrigorous approach to early termination because of large differences inflates the type I error, [69,70] and [71] such an approach in the face of small or no differences can inflate the type II error. Some designs allow early termination of apparently negative trials while maintaining high power to detect reasonable differences if they do exist. DeMets and Ware [72] proposed an asymmetric design that allows early trial termination not only when the experimental therapy offers large advantages but also when the new therapy appears equivalent to or worse than the standard. Whitehead and Stratton [73] developed a design based on similar considerations. Ellenberg and Eisenberger [74] showed that a two-stage design in which the study is terminated with a negative conclusion if the experimental therapy is producing outcomes equivalent to or worse than the standard therapy at the end of the first stage affects power only minimally. This type of design was further studied by Wieand and Therneau, [75] who determined the sample sizes required to maintain power at the nominal levels, and by Thall and co-workers, [76] who investigated optimal relative sizes for the two stages.

Sequential designs are desirable because they permit valid interpretation of significance levels even though a trial does not complete its full accrual. Their impact may differ depending on the particular clinical trial situations. When observation of the end point may be substantially delayed (e.g., survival time is the outcome of interest and average survival time is a year or more from study entry), a sequential design may not affect patient accrual, which may be almost complete by the time sufficient events have been observed to justify an interim analysis. Nevertheless, a sequential approach may permit earlier dissemination of study results, which may affect treatment decisions for others. For trials studying varying durations of therapy, earlier availability of results may also affect the treatment of patients in the trial itself.

### Factorial Designs

In a factorial design, two or more questions are addressed in the same cohort of patients by multiple randomization. For example, if a surgical question and an adjuvant chemotherapy question are of interest in a given population, a double randomization allocates the patients to treatment as shown in Table 18-4.

| Adjuvant therapy | Surgical technique A | Surgical technique B | |
|---|---|---|---|
| Therapy A | n/4 | n/4 | n/2 |
| Therapy B | n/4 | n/4 | n/2 |
| Total | n/2 | n/2 | n |

**TABLE 18-4. FACTORIAL DESIGN FOR RANDOMIZED CLINICAL TRIAL**

In Table 18-4, n is the total number of patients to be studied. If the effect of each factor can be considered to operate independently of the other (i.e., the difference in efficacy of the adjuvant therapy regimens does not depend on which surgical technique is used and vice versa), each question can be evaluated by collapsing over the categories of the other question. Thus, the n patients accrued may be used to evaluate two maneuvers rather than one. Factorial designs can improve the efficiency of clinical trials in situations in which the assumption of independent effects is reasonable. This assumption must be carefully considered; if the data cast doubt on its validity, categories are not collapsible, and the power of the study to detect differences is drastically reduced. If one of the maneuvers to be tested affects the administration of the other (e.g., if both questions involve cytotoxic drugs, and the doses may vary among the four possible combinations), the assumption of independence is clearly violated, and a factorial design is not appropriate. An example of factorial design is the National Wilms' Tumor Study III, in which early stage patients were randomized between postoperative radiotherapy and no postoperative radiotherapy and also between two different chemotherapy regimens. [77] Another example is a study of adjuvant therapy for colon cancer, in which patients were randomized to receive or not receive chemotherapy and to receive or not receive immunotherapy.[78] Various aspects of factorial designs are discussed by Byar and Piantadosi [79] and by Brittain and Wittes. [80]

### Equivalence Trials

An equivalence trial (sometimes called a *noninferiority trial*) is a phase III trial whose purpose is to demonstrate that a new treatment is no less efficacious than a standard treatment. This is important if the new treatment is clearly more desirable than the standard in some other way (e.g., less toxic, less invasive, less expensive, or more convenient). In such a case, one would prefer the new treatment as long as efficacy was not reduced.

The essential difference between an equivalence study and other clinical trials is that in an equivalence study, one is hoping to conclude that the two treatments are equally efficacious, but in the typical clinical trial, one is hoping to show that one treatment is more efficacious than the other. Enough patients must be entered into an equivalence study to demonstrate that a negative result is convincingly negative. A result of “no significant difference” is not sufficient, because one can easily assure such a result by designing a study too small to detect a credible potential effect. In general, for these types of studies, the preferred design is one in which the minimal difference of interest (usually denoted as d) is specified as the drug effect under the null hypothesis, and the alternative hypothesis is that there is no difference. Thus, the treatments cannot be accepted as equivalent unless one can rule out with high probability that the difference in effect is no greater than d. Sample sizes for equivalence trials are usually larger than those in difference-seeking trials to protect against adopting new approaches that are less efficacious than current practices. In circumstances in which a positive effect has not been documented for either treatment, or effects are not consistently seen or vary widely in size, an equivalence study will usually not be informative. [81,82] and [83] Sample size considerations for equivalence studies are discussed by Makuch and Simon [84] and Blackwelder.[85]

### Treatment Plan

The treatments to be delivered on protocol should be precisely and thoroughly defined to promote uniformity of conditions throughout the experiment. Variations in therapeutic intervention can reduce or destroy the interpretability of the results. All aspects of therapy should be set forth, including surgical procedures to be used and supportive care guidelines. Provisions for treatment modifications in the event of toxicity should be specified. In complex protocols, a scheme that shows the temporal relationships of chemotherapy and other treatment modalities from study entry through various treatment phases (e.g., induction, consolidation, or maintenance) to discontinuation of therapy is particularly useful. Schematics quickly convey information to medical personnel not directly involved in the development of the protocol. For the patient's safety, however, schematics must accurately and unambiguously describe medication prescriptions to those individuals actually delivering patient care.

### Drug Information

The drug information section serves as a reference for participants by supplying specifics about the mechanism of action, animal and human toxicology data, and pharmaceutical information for each of the drugs used in the clinical trial. This information is included to ensure consistency of preparation and administration of drugs by the various house staff, nurses, and pharmacists who are responsible for these activities, and it is an important quality control and safety measure. The protocol is often the only source of information for investigational drugs, which are not included in standard pharmaceutical references.

### Criteria for Evaluating Treatment Effect

The parameters for assessing the effects of treatment on individual patients are generally referred to as *end points*. An end point is a medical event that may represent benefit (e.g., complete remission) or harm (e.g., relapse, death). The results of the clinical trial are based on analyses of the accumulation of end point assessments, the criteria for which were predetermined by the investigator. A well-constructed protocol incorporates end points that are objective, practical, and

relevant to the clinical situation under study. By defining end points, the researcher indicates precisely which measures of treatment outcome reliably meet the objectives of the protocol. These objectives assist in clarifying what clinical and laboratory data need to be obtained during the trial and provide the basis for statistical analysis.

In phase I trials of classic cytotoxic agents, the end points are the degree and duration of changes in organ function after exposure to the experimental agent. It is useful to include in this section of the protocol a table that defines increasing degrees of toxicity for various organ systems and indicates the level of toxicity that is deemed unacceptable. In the interest of standardized reporting as well as accuracy, precision, and completeness, the NCI Common Toxicity Criteria version 2.0 should be used.[86] Phase I trials of newer anticancer agents that are intended to modulate or inhibit a specific cellular target associated with the malignant state may not require escalation to DLT, as the optimally effective dose may be significantly lower than the MTD for normal tissues. For these trials, end points are related to assessment of the specific modulation or inhibition being sought.

Phase II end points (and subsidiary phase I end points) are concerned with the evidence for and duration of response to the investigational agent. Complete response has been widely defined as the total disappearance of all clinically detectable malignant disease for at least 4 weeks, and partial response as a 50% or greater decrease in the sum of the products of the longest perpendicular diameters of all measurable lesions, with no increase in size of any lesions and no appearance of new lesions. Progressive disease denotes appearance of new lesions or enlargement of existing ones. [87] It has been suggested that response assessment might be derived from unidimensional tumor measurements instead of the conventional bidimensional method. [88] This concept has been incorporated into the new Response Evaluation Criteria in Solid Tumors guidelines in an attempt to simplify and standardize phase II methodology. [89]

In phase III trials, in which an untoward event (e.g., death, progression, or relapse) is the end point of interest, investigators generally find it useful not only to tabulate how many patients experienced that event but also to measure the time elapsed from entry to end point. This calculation provides a more sensitive basis for comparing the therapies because additional information is incorporated into the analysis. [90]

Because untreated malignancies are almost always fatal, the ultimate merit of a therapy resides in its ability to prevent the patient's death. Thus, a variable of unarguable interest is survival time; this end point, however, is often an impractical basis for evaluating treatment because death from disease may be considerably removed from the onset of therapy, and results of the trial may be correspondingly influenced by intervening events. Alternative end points are often chosen that are presumed to be early signals of long-term survival, such as the disappearance of detectable tumor or the absence of metastases at 3 years. These alternative end points may not always reflect survival, however, as when salvage therapies are effective despite prior treatment failure; the influence of an adverse outcome during the first treatment is abrogated by the successful second treatment. Demonstration of patient benefit in terms of survival is hampered not only by the logistics of follow-up but also by problems in analysis introduced when subsequent therapies are not uniform.

Because of the problems alluded to in the preceding paragraph, an end point widely used in trials of childhood malignancies is event-free survival. An *event* is defined by Mastrangelo and associates[91] as “the first occurrence of the major events that represent initial treatment failure: failure to achieve remission (i.e., death in the induction period or nonresponse), relapse at any site after achieving remission, and death in remission without preceding relapse.” This end point, also called *failure-free survival* or *time to first event*, is meaningful for studies in any disease population, but it is especially appropriate for trials in which most patients achieve remission and many achieve long-term survival. Mastrangelo and co-workers[91] urged that event-free survival always be evaluated in studies of pediatric acute lymphoblastic leukemia, along with any other end points believed to be of interest, to enhance the comparability of study results in this population.

Ancillary end points may pertain to quality of life; these are frequently highly subjective and are not easily quantified. An excellent review of methodologic issues in assessing these end points is given by Aaronson, [92] Testa and Simonson,[93] and, from a statistical perspective, by Cox and colleagues[94]; an overview of issues in the specific context of cancer trials is presented by Aaronson.[95] Increasingly important in studies of children are end points regarding adverse effects of treatment, such as the occurrence of second malignancies, growth disturbances, and neuropsychologic impairment attributed to therapy. [96,97,98 and 99]

**Clinical and Laboratory Data to Be Accessioned**

The data set required for determination of eligibility and evaluation of treatment effect must be presented in the protocol. This set includes pretreatment, on-treatment, and posttreatment evaluations, indicating specific clinical and laboratory assessments and their timing. These schedules are often presented in tabular form. In comparative trials, the frequency and nature of these assessments must be identical for the regimens being compared to avoid an unbalanced increase in the likelihood of detecting real or chance differences resulting from disparities in medical surveillance.

Clinical trials are also used to provide systematic information about the natural history of the disease independent of therapeutic intervention. [90] For example, patient characteristics are recorded at presentation and are subsequently correlated with outcome. The details of acquiring such data should be included in this section of the protocol.

**Statistical Considerations**

Statistical considerations for each objective of the study are included in the protocol. The estimated number of patients required for assessment of the primary and secondary end points, the anticipated rate of patient accrual, and the expected duration of the trial (including follow-up) are given along with the description of the proposed analysis of outcome data. In planning the study, the availability of patients should be documented whenever possible to determine whether study objectives are realistic. Documentation may be based on accruals to previous protocols for a similar patient population or on data from surveys of collaborating institutions that established the frequency of the required patient characteristics. Conducting a study that accrues too few patients and thereby provides uninterpretable results is a major waste of resources and is inappropriate from the perspective of a patient who agrees to participate on the understanding that the study will provide useful information. Similarly, accruing more patients than are necessary to provide accurate results may lead to patients being subjected unnecessarily to procedures not part of normal care and is also clearly a waste of resources. The central nature of these considerations necessitates close collaboration with experienced biomedical statisticians from the outset.[100]

**Informed Consent**

All research projects that involve human subjects and are conducted or supported in part or entirely by the U.S. Department of Health and Human Services are subject to regulations regarding the protection of those subjects (see Chapter 48).[10] The Office for Human Subjects Protection, formerly the Office for Protection from Research Risk, is the government agency charged with ensuring that all research is conducted according to these regulations.

Documentation that research subjects have given prior informed consent to participate is an absolute requirement of cancer clinical trials supported by federal funds. The federally required elements of informed consent are presented in Table 18-5 in the checklist format used by NCI staff to evaluate these documents.[13] In addition, templates containing these elements are available at the NCI's CancerTrials website. [101,102]

**TABLE 18-5. INFORMED CONSENT CHECKLIST: REQUIRED ELEMENTS**

Every clinical trial should include a sample informed consent document, which can be reviewed by the NCI or other appropriate agency for completeness and also can be used by individual sites in a multicenter trial to construct the consent document to be used at that institution. The local version of the consent should not differ substantially from the sample document and must contain all federally required elements.

Federal law prescribes additional protections specifically for children who are subjects of clinical research. [103] Children are defined as persons who have not reached the legal age of consent to treatments or procedures involved in the research; legal age is determined by the applicable law of the jurisdiction in which the research is conducted. Informed consent must be obtained from the parent or guardian before research procedures can begin. The informed consent of one parent is usually adequate if the child is being enrolled onto a study from which he or she may receive direct benefit from the research, such as in a therapeutic trial. In addition to the informed consent of the parent, investigators wishing to enroll children onto clinical trials must obtain assent from the child in a manner appropriate for the child's age. Although the process of obtaining informed consent can be difficult and time consuming, it is critical to the ethical conduct of clinical research.

## MANAGING THE CLINICAL TRIAL

### Registration

Every study patient should be formally registered as a study participant before receiving any protocol-directed intervention. Registration for a nonrandomized study simply refers to the entry of a patient's name or hospital number into a paper or computer log. Pretreatment registration ensures that all patients who begin treatment can be identified for reporting purposes at the end of the study. In addition, the process of registration can be used to verify that the patient meets the eligibility criteria. Registration is important, even in studies conducted within a single institution, as a quality control measure to prevent the inadvertent loss of “problem patients” (e.g., early deaths and disease progressions or refusals of further treatment after minimal therapy) from the reporting process. In randomized studies, the process of randomization usually ensures the recording of all patients entering the study. Studies in which randomization follows an interval during which all patients are treated uniformly (e.g., a study of maintenance therapy in which all patients receive the same induction therapy) should require registration before the initial treatment interval to identify any selection patterns that may affect the generalizability of trial results.

### Randomization

The purpose of randomization is to avoid systematic bias in the allocation of patients to treatment in comparative trials. A *bias* is the effect on a study result of some systematic aspect of study design, data collection, or analysis that is unrelated to the actual effect of the treatment under study. For example, a comparison of a medical treatment with a surgical procedure in which treatment assignment depends on a patient's health status (e.g., patients who receive medical treatment are those whose poor condition precludes surgical procedures) is biased. The surgically treated patients are in better shape from the beginning; if their outcomes are better, one has no way to know how much of this superiority is attributable to treatment. This may seem an extreme and obvious example of bias that can be avoided by randomizing patients to treatment. Bias can find its way into even the most meticulously randomized trial, however.

Bias will almost surely be introduced if an investigator knows the next treatment assignment before deciding whether to offer participation in the study to the next available patient. It is important, then, to select a method of randomization that does not permit prediction of treatment assignments. Ad hoc methods, such as assigning alternate patients to each of two treatment groups or assigning patients on the basis of birth date (e.g., odd numbers assigned to one group, even numbers to the other), do not provide adequate protection against predictability and should be avoided. [104]

Perhaps the most widely used mechanism is the random number table in published form or generated by a computer program. In using a published table, one begins at some “randomly chosen” entry in the table and uses the sequence of numbers beginning with that entry to determine successive treatment assignments. For example, in the case of two treatment groups, the parity of the number (odd or even) can determine the associated treatment assignments. Alternatively, computer programs that generate series of random numbers may be used to generate treatment assignment lists, which must not be accessible to physicians participating in the trial. (Computer-generated random numbers cannot be said to be truly random, because they eventually repeat. The number of patients on any given trial is small compared with the cycle of repetition, however, making computer-generated numbers acceptably random for practical purposes.)

When subgroups of patients with identifiably different prognoses are studied in the same trial, a stratified randomization plan may be considered. The purpose of stratification is to ensure that patients with a better or worse prognosis are not overrepresented in a particular treatment group. The simplest way to stratify at randomization is to generate a separate allocation list for each subgroup. Thus, if three factors, each with two possible levels, are used for stratification, one needs eight separate lists, one for each possible combination of factor levels. An alternative method involves determining for each new patient the treatment allocation that will result in the best overall “balance” of factors of interest. Patients are directly assigned to a treatment (deterministic method) or are assigned to treatment by a random mechanism that yields a high probability (but not 100%) that they will be allocated to the treatment resulting in the best balance (random method). Detailed calculations are required to determine the balance for each possible allocation; a computer program is the only practical method when more than two or three stratification factors are used. This method of treatment assignment, usually referred to as *adaptive allocation*, has become popular in multicenter trials despite its complexity. Adaptive allocation methods have been described in several reports. [105,106,107 and 108] Deterministic adaptive allocation is not actually a form of randomization because the allocation is determined by the distribution of prognostic factors for the new patient and for the series of patients already entered. Nevertheless, the allocation is, for all practical purposes, not predictable because of the complexity of the required calculations, and it may generally be considered an unbiased allocation, even though it is not strictly random.

To protect the validity of randomization, investigators must establish procedures that are not susceptible to “tampering.” Envelope randomization is less desirable than telephone randomization, for example, because envelopes can be opened and resealed, and the decision to randomize or not to randomize a patient may depend on the contents of the envelope. A randomization list, monitored by a central data manager or a randomization clerk and accessible only in response to a request to randomize a specifically named patient, provides greater security.

The timing of randomization is important. Patients should generally be randomized as close in time as possible to the point at which the treatment programs to be compared actually begin. When a substantial interval occurs between randomization and initiation of the treatment to which the patient was randomized, some patients do not receive the assigned treatment. Some change their minds; some experience changes in status, making the treatment no longer appropriate; and some die. If these patients are included in the analysis, the comparison will be diluted because some patients will not receive the assigned treatment. Their exclusion, however, can introduce bias if the nondelivery of treatment is related to the treatment assigned. For example, patients with poorer prognoses may be more likely to refuse or to become unable to tolerate the more intensive treatment. This issue is especially problematic in studies of maintenance therapy, in which all patients receive the same induction therapy; the induction interval may be as long as 4 months, and many patients may never enter the maintenance phase because they die, their disease progresses, or some other circumstance intervenes. To avoid serious problems in analyzing and interpreting the study data, the preferred procedure is to register patients before induction therapy and to explain that randomization to maintenance therapy will occur in the future. Informed consent, followed by randomization, takes place only after successful completion of induction. Some patients may refuse randomization at this time, but the analysis of all who do agree to participate is unaffected by the problems delineated earlier.

The randomized consent design proposed by Zelen [109,110 and 111] provides for randomization before informed consent, so patients approached to participate in a trial are told what their treatment assignment is. Patients who refuse the allocated treatment are still followed and analyzed as part of that treatment arm. This design was motivated by the belief that providing the treatment assignment as part of the informed consent procedure would make physicians and patients more comfortable and would enhance participation in trials. Early experience with this design, however, has not generally resulted in improved efficiency in completing trials, and ethical concerns about the changed nature of the informed consent process have been raised. [112,113]

### Data Collection

The design of data forms is not a trivial task. It requires the input of a clinician, statistician, data manager, and computer programmer. All data items necessary to meet the objectives of the protocol must be included, and those unlikely to be of interest should be eliminated. The designers must ensure that items are unambiguously presented, coding procedures are consistent and straightforward, the form is structured for maximal efficiency in entering and keying the data, and the format of the data allows efficient analysis.

Determining the required data items is not always simple. One must go through the protocol step by step and carefully consider what information must be collected to answer the questions addressed. It is important to be selective. The collection and computer entry of excessive, nonessential data waste valuable hours.

Composing a perfect data form on the first attempt is almost impossible. Ideally, the forms should be “piloted,” perhaps on patients who are not participants in the study, before they are used to collect study data. Only by actually using the forms can errors and ambiguities be discovered. Instructions should be contained within the form to the extent possible. For complicated forms, a special coding manual may be required. Institutions or cooperative groups often develop general instructional manuals for completing data forms that are used in a variety of studies. The advantage of shortening the form by removing instructional material must be balanced against the advantage of having the instructions immediately available.

Forms should be designed with ease of completion as a primary consideration. Errors are more likely to be made when the form is filled out than at any other point in the data management process. Ease of data entry is also important, and experienced data managers and data entry personnel should be consulted when new forms are designed. The efficiency of forms with regard to computer programming and data analysis is also important, but it is secondary to the previous considerations.

A schedule for collection of data forms should be established, publicized to the investigators, and enforced by the data management offices. Delay in completing the data forms increases the potential for errors and for missing data that may become irretrievable. Because data managers are often nurses with clinical and administrative responsibilities, sufficient time must be allotted for them to complete and submit forms on the required schedule. Proper monitoring of a study cannot be reliably accomplished without a continually current database.

Advances in computer technology have radically changed data management practices. Almost all clinical trial data are now entered into computer databases for further analysis. Data may be collected on paper forms and sent to a central site for processing, or may be entered directly into electronic forms on disks or via an Internet or other remote application. Remote data entry has the potential to drastically decrease the amount of paper consumed during the conduct of clinical trials, but implementation has proved challenging to investigators comfortable with paper-based systems. Nevertheless, electronic submission of data appears to be inevitable as long as the quality of the data submitted is maintained or improved.

**Quality Control**

*Quality control* refers to all the checks and reviews of data over the course of the study that are designed to make sure the protocol is appropriately followed and the data submitted are accurate. Quality control considerations should affect every step of the protocol, from patient entry through final follow-up. Much of the responsibility for quality control during the course of the study falls to the central data management personnel.

Checklists should be used at the time of patient registration or randomization to ensure that the patient actually is eligible and willing to participate before he or she is formally entered on the study. Data entry procedures should be developed to minimize errors in transposing data from the form to the computer file. Many data centers require two independent keyings of every form, with software designed to catch discrepancies. Many centers have also developed software to simulate the data form on the computer screen, with the cursor moving automatically from one field to the next, to increase the efficiency of entry and to control the error rate. Computer programs must be written (and thoroughly pretested) to detect errors and missing information in the data entered. A system must be devised to notify physicians or data managers about errors, to request corrected or updated information, and to flag persistent errors. For multicenter studies or single-institution studies with multiple participants, it is worthwhile to conduct training sessions for staff who are responsible for completing data forms and for submitting the forms to a central office. Complex protocols often require initial training sessions for surgeons, radiotherapists, pathologists, and others who may need more instruction in the experimental procedures than can be reliably transmitted in the written protocol.

Many aspects of the protocol must be subject to medical review. For example, when radiotherapy is an important part of an experimental treatment program, a centralized quality assurance review of the port films is mandatory to ensure that the treatments are administered according to the protocol. Review must be prompt, especially at the beginning of a study, so problems can be corrected before they affect a large proportion of the study population. Pathology and surgical reports must be reviewed for final determination of patient eligibility. Again, these reviews must be performed promptly because a high ineligibility rate signals the need for modification of the patient entry procedures. Reports of responses, relapses, or other events of interest may also require review. The responsibility for data reviews is usually shared among the study chairperson, data management staff, and treatment specialists.

A series of papers on quality control issues in cancer trials provide some useful insights and examples. [114,115,116,117] and [118] Neaton and colleagues[119] looked at training and data verification procedures as part of the overall quality assurance effort. Peduzzi and co-workers [120] and Miller and associates[121] looked at costs and benefits of central laboratories in multicenter trials. More recently, a position paper on quality assurance in multicenter trials was published by a special committee of the Society for Clinical Trials. [122] The paper addresses a variety of issues in assuring high-quality clinical trials data and is one of the few published sources of recommendations in this important area.

**Follow-Up**

For patients entered on phase I and phase II trials, follow-up is typically limited to the period during which the end points of primary interest (e.g., acute toxicity and tumor response or progression) may be seen. Phase III studies, with the goal of defining optimal treatment strategies, require more extensive follow-up. Ideally, all patients enrolled in phase III clinical trials of cancer treatment should be followed throughout the remainder of their lives, although this is not always feasible due to limited resources. Extended follow-up has two major purposes. The first is to maintain a check on the treatment comparisons by detecting any late crossing of survival curves and obtaining better estimates of possible cure rates. The second is to detect late adverse effects of the treatment that may not be evident when trial results are initially reported. In pediatric trials, late adverse affects include second malignancies, sterility, and cognitive dysfunction. Although data to demonstrate that these effects are sequelae of radiotherapy or chemotherapy rather than the disease process itself are often lacking, a strong biologic basis frequently exists for attributing these effects to cytotoxic therapy. Follow-up forms should specifically request information about known or suspected adverse effects of the therapies used and the disease studied and information about all other adverse effects noted, regardless of whether an association between the effect and prior treatment appears plausible.

The desirable frequency of follow-up reporting varies with the time since study entry. Patients should be assessed frequently, preferably three or four times a year, as long as the study is in an active stage (i.e., before reporting of results). Without frequent follow-up, one cannot monitor study results reliably. For example, extreme differences observed early in a study might lead to consideration of early termination. Without current follow-up on all patients, one cannot know to what extent the observed difference may be an artifact of delayed reporting.

After study results have been reported, a large proportion of surviving patients may be expected to be long-term survivors. It may then be reasonable to request follow-up reporting only on a semiannual or yearly basis, with the understanding that any deaths, relapses, or adverse effects should be reported immediately.

**Data Monitoring and Interim Analysis**

The data from clinical trials must be regularly monitored to check for problems in implementing study procedures, for unexpectedly severe toxicity that may require modification of doses/schedules or even termination of the study, or for early evidence related to treatment effects that may also require early termination. Phase III studies should use sequential designs, as discussed in the section on sequential design, to account for the possibility of early termination because of strong early results. NCI-supported clinical trials cooperative groups have implemented formal data monitoring committees to evaluate interim phase III results on a regular basis and to make recommendations to the group chairperson regarding the conduct of ongoing studies. [123] The voting members of these committees are generally clinicians and statisticians with no direct involvement in the leadership of the trial under consideration, to avoid potential financial or professional conflict of interest during decision making. A majority of the voting members of the committee is not affiliated with the group; laypersons such as cancer survivors or patient advocates are generally included, but all members are expected to see themselves as representing the patients' interests. The committee reviews the accumulating data on a schedule related to the trial design and may recommend modifications to the study design (including early termination) based on the interim results. Knowledge of the interim data is restricted to the committee and is considered confidential, because when all trial participants have access to the accumulating results, nondefinitive trends in the data may engender reluctance in some physicians to continue entering patients on the trial. [124] Discussion of a variety of practical issues in data monitoring can be found in published proceedings of an international workshop on this topic. [125]

A statistical monitoring procedure known as *stochastically curtailed testing* evaluates the probability of reversing the currently observed result. [126,127] and [128] For example,

if most of the required number of patients have been entered onto a trial, and if the results of the arms have been similar, one may be able to show that, under a range of reasonable assumptions about what will happen in the remaining patients to be accrued, the probability that one treatment will be shown statistically superior to the other is negligible. This calculation may provide the basis for terminating the study early, even when a sequential design is not established initially.

General issues in study monitoring and interim analysis are discussed by DeMets, [129] Fleming and DeMets,[130] Green and colleagues,[124] Geller and Pocock,[131] Fleming,[132] and Souhami and Whitehead.[133]

## ANALYSIS

The most efficient, most sophisticated statistical analysis cannot compensate for major errors in the design or conduct of a clinical trial. This does not, however, diminish the importance of proper selection and use of analytic procedures. Before discussing specific procedures useful in cancer clinical trials, one should consider some general issues relevant to the analysis of clinical trial data.

### Avoidance of Bias

The most common cause of bias at the analysis stage of clinical trials is the improper exclusion of patients from analysis. This exclusion can affect not only comparisons of treatments but also estimates of the effect of a single regimen. A good rule is that patients who meet the eligibility criteria, who are entered on the study, and who begin treatment should be included in the analysis of study data. This approach is known as *intention-to-treat*, because analysis includes all patients intended to receive the assigned treatment, whether or not it was ultimately fully administered. [134]

An unbiased estimate of the probability of response to a treatment regimen in a given patient population requires the inclusion of all patients in that population who have been treated with the regimen. Problem patients, such as those who die or who refuse further therapy after only one or two doses of drug, are representative of some fraction of the overall population who may be eligible for this regimen but who will not benefit from it because their disease is too far advanced or because they find the treatment intolerable. When such patients are classified as “inevaluable” and are excluded from study analysis, the resulting response rate will overestimate the proportion of patients in the target population who would actually show tumor regression if treated with the regimen. A response rate has a clear meaning only if the numerator is the number of patients who respond and the denominator is the total number treated. The proportion of responders among patients who receive “adequate” treatment (as defined by each investigator) may have some secondary interest but is not readily interpretable by the medical community at large.

In a randomized study, improper exclusions can clearly bias the treatment comparison. Consider a population of children who have achieved complete response to induction therapy and are then randomized to receive either maintenance therapy or no further treatment. The protocol requires that maintenance therapy begin within 14 days of completion of induction therapy. For some patients, initiation of maintenance therapy is delayed; several other patients become sicker or die in this interval; and a few patients refuse maintenance therapy despite their prior agreement. Some investigators exclude such patients from analysis on the basis that the maintenance therapy may be ineffective if delayed too long (and certainly cannot be effective if not given). It may also be true, however, that patients with poorer prognoses are more likely to present these kinds of problems. Moreover, patients randomized to “no further therapy” are not excluded on the basis of treatment delay or inadequate courses of therapy. Thus, the exclusions may bias the comparison in favor of the maintenance treatment. Additional discussion of this topic is provided by Gail.[135]

### Multiple Comparisons and Subsets

If a box contains 19 black balls and one red ball, the probability of selecting the red ball on any single draw is 1 in 20 or .05. The probability of selecting the red ball at least once is .23 if one draws five times (replacing the ball each time) and .40 if one draws ten times, however. In a clinical trial with the data subjected to multiple tests of hypothesis, the probability of at least one spuriously positive result increases rapidly beyond the nominal .05 level of each individual significance test. No totally satisfactory way around this dilemma exists. It does not seem realistic to limit the exploration of data obtained at great expense, nor does it seem reasonable to require that all tests be done at strict significance levels to ensure that the overall significance level be protected at the cost of severely reducing the power to detect important effects. An intermediate approach may be to consider all questions other than the primary focus of the trial as exploratory questions. Tests can be done at the usual .05 or .01 level, but a significant result may be interpreted as a suggestive observation requiring confirmation rather than a definitive result. This approach has been suggested by several investigators. [136],[137] and [138]

An important example of the multiple comparisons problem in clinical trials is the analysis of data within patient subsets. To speculate that treatment effects may be limited to or more pronounced in some subgroups of patients is reasonable, but confirming this speculation is difficult without extraordinarily large sample sizes. The more subsets considered, the greater the chances that either a uniformly ineffective therapy will appear effective in one or more subsets or a uniformly effective therapy will appear ineffective in one or more subsets. In randomized studies, one can test for the significance of interactions between treatment and covariates (i.e., differential effects in subsets beyond what is expected by chance). However, the power for detecting interactions in a clinical trial of moderate size is low. “Qualitative interactions” (i.e., beneficial effect in one subset, harmful effect in the other) are of special concern, but even these are difficult to detect reliably. Thus, when apparent differences in subset-specific treatment effects are observed in a clinical trial, one cannot (except for extreme differences) determine with confidence whether they are real or spurious.[139] The most sensible approach seems to be to base conclusions on the overall result. The overall result is the most stable, and the study population presumably is defined on the basis of an expected homogeneity of treatment effect. Differences in subset-specific treatment effects, if suggestively large, should be independently confirmed before they are accepted. Problems in interpreting data from trial subsets are discussed in more detail by Yusuf and associates [140] and by Assmann and co-workers.[141]

### Phase II Studies

Phase II studies generally are concerned with response rates. The observed response rate (i.e., number of responders per number of patients treated) is usually the statistic of primary interest. Although this is our best estimate of the true response rate—the proportion of patients who would demonstrate response in an infinite patient population—one cannot assume on the basis of findings from 30 to 40 patients that the estimate is precise. To evaluate how close the estimate is likely to be to the true response rate, a confidence interval is constructed around the estimate. A confidence interval can be defined as the set of possible rates that includes, with specified probability, the true response rate.

The most widely used method to calculate a confidence interval for a proportion p observed in a sample of size n is based on the normal approximation to the binomial distribution. The formula for calculating the upper and lower limit of the confidence interval for p is given in Table 18-6A. The point on the tail of the standard normal curve (i.e., centered at 0, standard deviation of 1) beyond which only a (specified) small portion of the distribution lies is z. A few commonly used z values are given in Table 18-6B, and an example of the actual calculation of confidence limits is given in Table 18-6C. Although this method is simple, it is based on an approximation that is reasonably accurate only when p is in the middle range ($0.3 < p < 0.7$) and when n is approximately 30 or more. For smaller sample sizes, or when proportions closer to 0 or 1 are estimated, one can use a better approximation that is only slightly more complicated to calculate ( Table 18-6D).[142]

(A) Approximation to upper and lower confidence limits

$$p \pm z\sqrt{p(1-p)/n}$$

(B) Commonly used $z$ values

| Confidence interval | $z$ |
|---|---|
| 99 | 2.58 |
| 95 | 1.96 |
| 90 | 1.645 |

(C) Example of calculating confidence interval

Number of patients (n) = 30
Observed responses = 9
Observed response rate (p) = 9/30 = 30%
For a 90% confidence interval, $z = 1.645$.
Confidence limits are calculated as follows:
$= 0.30 \pm 1.645\sqrt{(0.30 \times 0.70)/30}$
$= 0.30 \pm 0.14$
$= (0.16, 0.44)$

(D) Better approximation to confidence limits

$$p + A/2 \pm z\sqrt{p(1-p)/n + A/4n}/(1 + A)$$

where $A = z^2/n$.

**TABLE 18-6. CALCULATING CONFIDENCE LIMITS FOR PROPORTIONS**

The estimation of survival time parameters is more complex. If all patients have died, so that all survival times are known exactly, one can directly calculate the median survival and the proportion surviving at various times. The median of the observed survival times is the halfway point in an ordered listing of all survival times. It is preferable to the mean as a central measure for this type of data because the mean can be drawn away from the center of the distribution by one or two large observations, as can be seen in Table 18-7. This example also demonstrates that the central measure, whether median or mean, is often insufficient to summarize the data adequately. For the data in the table, the range of values is essential to a useful description.

| |
|---|
| Observed survival times 1, 1, 1, 1, 1, 2, 2, 2, 2, 2, 3, 3, 3, 4, 5, 5, 5, 20, 30 |
| Mean survival time 5.5 |
| Median survival time 2 |

**TABLE 18-7. CENTRALITY ESTIMATES FOR SURVIVAL TIMES (MONTHS)**

When data are censored (i.e., some patients are still alive, so their survival times are known only to be longer than the current follow-up time), estimation of survival parameters is more difficult. Some parameters can still be measured directly. For example, if more than 1 year has elapsed since patient entry was terminated and if follow-up is current on all patients, the proportion of patients surviving for 1 year can be directly estimated by dividing the number of patients who survived for 1 year by the number of patients who entered the study. If the censored survival times are all in the upper half of the distribution when the times are ordered, the median is still the central observation.

When some data are censored at early points in the study, however, we can no longer estimate median or year-by-year survival rates by ordering the observations and counting. In these situations, life-table methods are required to provide valid and maximally informative estimates. The Kaplan-Meier method, which provides probabilities of survival at each point in time at which a death occurs, is probably the most commonly used in cancer studies and should always be used when the number of deaths is small.[143] The other type of procedure, often referred to as the *actuarial method*, calculates probabilities at fixed points in time (e.g., 6-month intervals).[144] With this procedure, some information is lost, because the ordering of deaths within any given interval is ignored. If the number of deaths is large, however, and the size of the interval is chosen sensibly, the loss of information is not serious. This procedure requires less calculation, but with the availability of computer programs to perform these procedures, complexity of calculation has become less of an issue.

Life-table survival probabilities are frequently presented graphically as survival curves. Figure 18-1 shows the Kaplan-Meier plot of survival probabilities for the data set shown in Table 18-8. These hypothetical data represent a clinical situation in which 30 patients were entered on a study over a period of 300 days, with follow-up continuing until day 365. The plot steps down at each point in time at which a death was observed. The tick marks on the plot correspond to the survival times with a plus sign in Table 18-8, indicating data censoring at those points. For example, the survival time for patient 5 in Table 18-8 is given as 326+ days. The experience of this patient is represented by the far right tick mark on the survival plot. The estimated median survival and probabilities of survival at particular points may be approximated from the plot or, more easily, from the table that produced the plot (Table 18-9). In this case, the median survival is 145 days; the probabilities of 3-, 6-, and 9-month survival are 63%, 45%, and 21%, respectively. The actual calculation of Kaplan-Meier life-table probabilities is well described by Peto and colleagues [145]; both procedures are described by Lee.[146] Methods for calculating confidence intervals for survival parameters are discussed by Simon. [142]

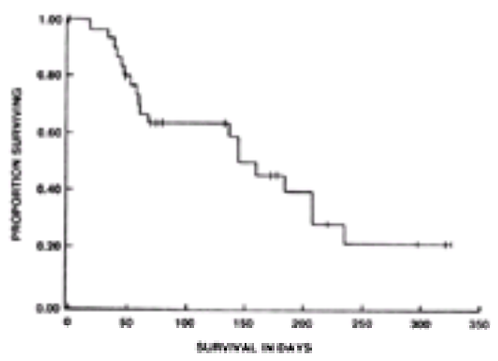


**FIGURE 18-1.** Plot of life-table probabilities. Tick marks indicate censored observations.

| Patient | Day of entry | Status at day 365 | Survival time* |
|---|---|---|---|
| 1 | 11 | Dead | 53 |
| 2 | 23 | Dead | 41 |
| 3 | 35 | Dead | 208 |
| 4 | 37 | Dead | 208 |
| 5 | 39 | Alive | 326+ |
| 6 | 44 | Alive | 321+ |
| 7 | 55 | Dead | 60 |
| 8 | 68 | Alive | 297+ |
| 9 | 79 | Dead | 137 |
| 10 | 85 | Dead | 188 |
| 11 | 86 | Dead | 160 |
| 12 | 91 | Dead | 335 |
| 13 | 94 | Dead | 48 |
| 14 | 104 | Dead | 145 |
| 15 | 139 | Dead | 145 |
| 16 | 144 | Alive | 221+ |
| 17 | 188 | Alive | 181+ |
| 18 | 191 | Dead | 58 |
| 19 | 193 | Alive | 172+ |
| 20 | 205 | Dead | 61 |
| 21 | 208 | Dead | 68 |
| 22 | 217 | Dead | 34 |
| 23 | 232 | Alive | 133+ |
| 24 | 248 | Dead | 45 |
| 25 | 261 | Dead | 18 |
| 26 | 282 | Dead | 39 |
| 27 | 285 | Alive | 80+ |
| 28 | 290 | Alive | 75+ |
| 29 | 295 | Alive | 70+ |
| 30 | 297 | Alive | 68+ |

*Plus sign (+) indicates a censored survival time.

**TABLE 18-8. PATIENT ENTRY AND SURVIVAL TIMES**

| Days from study entry | Proportion surviving |
|---|---|
| 18 | 0.967 |
| 34 | 0.933 |
| 39 | 0.900 |
| 41 | 0.867 |
| 45 | 0.833 |
| 48 | 0.800 |
| 53 | 0.767 |
| 58 | 0.733 |
| 60 | 0.700 |
| 61 | 0.667 |
| 68 | 0.633 |
| 137 | 0.588 |
| 145 | 0.543 |
| 145 | 0.498 |
| 160 | 0.452 |
| 184 | 0.396 |
| 208 | 0.339 |
| 208 | 0.283 |
| 295 | 0.212 |

**TABLE 18-9. KAPLAN-MEIER LIFE-TABLE PROBABILITIES**

Evaluating the effect of therapy on patient survival is difficult in the context of a phase II study. Objective tumor shrinkage is generally accepted as attributable to the treatment administered because the frequency of spontaneous tumor regression in untreated patients is thought to be low. Without a control group, however, one cannot demonstrate, except in dramatic circumstances, that survival benefit is attributable to a particular treatment. Although investigators can reasonably assume that a tumor response would not have occurred in an untreated patient, one cannot so readily assume that a patient who survives for a given number of months after treatment would have survived for a shorter period without treatment. (The exception is the dramatic circumstance of a substantial proportion of patients who achieve long-term survival and who have a disease that previously was uniformly fatal within a short interval.) Investigators reporting results of phase II studies have commonly compared the survival experience of responders with that of nonresponders and have viewed a prolonged survival of the responders as evidence of survival benefit attributable to the treatment. The fallacious reasoning behind this type of analysis has been widely discussed. [147,148 and 149] The fundamental problem is that an association between response and survival cannot be assumed to be causal. Patients show variable survival times whether they are treated or not; patients who are destined to survive longer because of more favorable baseline characteristics may be those who exhibit tumor shrinkage when treated with anticancer therapies. Thus, effects of treatment on survival may be reliably demonstrated only with the use of appropriate control groups.

## Phase III Studies

Most of the methods discussed in this section are directed at the comparison of treatment effects, which is the major objective of phase III trials. Other analyses are considered, however, such as the identification of prognostic factors. Estimates of survival and response parameters are obtained using the methodology described in the previous section. The larger sample sizes available in phase III trials permit more reliable estimates of these parameters.

The general approach in statistical testing is to consider a null hypothesis (no difference in treatment effect) against an alternative hypothesis (unequal effects). When the data demonstrate a sufficiently large difference in patient outcome, the null hypothesis may be rejected. The *p* value associated with the statistical test of the null hypothesis can be interpreted as follows. If the null hypothesis were true (i.e., if there were truly no difference in treatment effect), the probability of an observed difference as large or larger than this one would be equal to *p*. Thus, if *p* is small, the observed data may be considered sufficiently inconsistent with the null hypothesis to warrant its rejection. Failure to reject the null hypothesis does not demonstrate that the null hypothesis is true. If the sample sizes are large enough, however, failure to reject this hypothesis may indicate inconsistency with a true treatment difference that is of clinical importance.

### *Comparison of Proportions*

When the end point is binomial (i.e., only two outcome possibilities exist), the data can be represented in a 2 by 2 table, as depicted in Table 18-10A. Unless an unbalanced randomization scheme is used, a + b and c + d each approximately equal n/2. If no association exists between treatment group and the likelihood of a success, then a/(a + b) and c/(c + d) should be approximately equal.

(A) Representation of data

| | Treatment | | |
|---|---|---|---|
| Outcome | A | B | Total |
| Success | a | c | a + c |
| Failure | b | d | b + d |
| Total | a + b | c + d | a + b + c + d (= n) |

(B) $\chi$-square statistic with correction for continuity

$$\chi^2 = \frac{n(|ab - bc| - n/2)^2}{(a+b)(c+d)(a+c)(b+d)}$$

(C) Significance levels for the $\chi$-square statistic

| *p* value | $\chi^2$ |
|---|---|
| .10 | 2.71 |
| .05 | 3.84 |
| .025 | 5.02 |
| .01 | 6.63 |
| .005 | 7.83 |
| .001 | 10.83 |

**TABLE 18-10. c-SQUARE TEST FOR COMPARISON OF PROPORTIONS**

The most commonly used test of the null hypothesis of no association is the c-square test. This test approximates the distribution of the binomial outcome variable with a normal distribution (bell-shaped curve) and therefore should be used only when the sample size is large and the proportions of successes are neither extremely low nor extremely high. A good rule of thumb in the case of equal numbers in the two treatment groups is that the c-square test is appropriate when the total number of successes (a + c) is no less than 10 and no more than n – 10. The formula for the c-square statistic is given in Table 18-10B. The value n/2, subtracted inside the squared term in the numerator, provides an adjustment ("correction") to account for the application of normal distribution theory to binomial data. When the value of c-square is large, the null hypothesis may be rejected at a significance level that depends on the magnitude of the statistic. Some commonly used values are given in Table 18-10C; more extensive tables can be found in many standard textbooks.[150,151]

When the data are such that the c-square test may be inappropriate, the Fisher-Irwin test (Fisher's exact test) may be performed. This test requires more extensive computation, but it is available in many statistical software packages. Alternatively, extensive tables for use with this test have been published. [152] The calculations required to perform the Fisher-Irwin test are described by Fleiss. [54]

### *Comparison of Survival Times*

In many, if not most, phase III trials in cancer, the primary end point is survival. In comparing survival (or similar end points, such as event-free survival), the use of proportions is not entirely satisfactory. The construction of a table, as described in the previous section, using "alive/dead" as the outcome variable can be misleading if follow-up reporting is delayed longer on one treatment arm than on the other, such as occurs when one treatment requires more frequent contact with the clinic. If we standardize follow-up by modifying the outcome variable to "alive/dead at 3 years after study entry," we lose information on patients who entered the study fewer than 3 years before the analysis, and we gain no information from deaths after the 3-year point.

To improve the efficiency of survival comparisons, methods have been developed that use all the information available for every patient and allow comparison of the overall distributions of survival time, rather than focusing on a particular point in time. These methods also accommodate censored data. Two methods that can be recommended in most situations are the log-rank test and the test based on the Cox proportional hazards model. They are most safely implemented with a well-validated computer program. (Programs for these procedures are available in most large statistical software packages for mainframe computers and in many packages for microcomputers.) An excellent discussion of the actual mechanics of the log-rank test is given by Peto and colleagues [145]; Lee[146] discusses these and other methods for survival analysis in nontechnical terms and provides many excellent examples to illustrate the calculations. [146]

The often-used term *proportional hazards* means that the relative superiority (with respect to prolonging survival) of one treatment over another remains constant over time. The assumption of proportional hazards is the basis of the Cox procedure, and although it is not formally required by the log-rank test, marked nonproportionality of hazards severely limits interpretability of test results. Under many circumstances, hazards are not necessarily expected to be proportional. One example is the comparison of a highly toxic regimen with one that is less toxic. The more toxic regimen may result in more treatment-related deaths early in the study, but it may provide a better chance for long-term survival, even with the excess of early deaths taken into account. In these circumstances, an overall test of survival may obscure the appropriate interpretation of the data. In many instances, particularly with pediatric patients, treatment to improve the chance of long-term survival may be the appropriate strategy, even though the risk of early death resulting from acute toxicity may be greater. The additional early deaths may prevent the more toxic regimen from demonstrating a statistically superior survival advantage overall, however. An excellent discussion of this problem and suggested analyses to demonstrate the

nonproportionality of hazards is provided by Stablein and co-workers. [153]

### *Identification of Prognostic Factors*

The statistical literature concerning the identification of baseline factors associated with eventual patient outcome is extensive. The methodology most suited to determining which of a set of factors is important in predicting outcome is the regression model. These models are used to develop prediction equations for outcome, based on the values of the known factors. Because the outcome of interest in cancer studies is usually binary (e.g., response-nonresponse) or a time variable that may be censored, specialized models must be chosen that accommodate such outcomes. With a binary outcome, one is essentially trying to calculate the probability of the outcome of interest; with a time-interval outcome, one is trying to predict the length of survival or event-free survival. For binary outcomes, the procedure of logistic regression is probably the most appropriate technique for evaluating the relative importance of baseline factors. The Cox regression model is widely used for outcomes such as survival time, in which some of the data may be censored.

In building either type of model, parameters reflecting the contribution of each factor to the accurate prediction of the outcome are estimated. When these parameters differ significantly from zero, one can infer that the factors have some prognostic value. Of course, when many factors are evaluated, the probability that at least one will differ significantly from zero, even if none has any effect on outcome (i.e., the multiple-comparisons problem), is not negligible. Therefore, the results of these analyses must be interpreted cautiously. The multiplicity problem also extends to the selection of “cutpoints,” which are values of covariates used to divide the population for purposes of stratification and analysis, as noted by Altman and colleagues. [154] The significance levels resulting from these analyses depend on the sample size. Weak associations may attain significance when large data sets are analyzed; conversely, strong associations may not be demonstrated at conventional levels of significance in small samples. The identification of several “significant” prognostic factors is therefore no guarantee of a highly predictive model. Simon and Altman[155] discuss the many pitfalls in the evaluation of prognostic factors and propose a hierarchy of prognostic factor studies similar to that for clinical trials. Phase I studies are the initial exploratory studies that identify new factors possibly associated with outcomes of interest. Phase II studies focus on categorizing patients according to risk levels, and phase III studies pre-specify and test hypotheses regarding treatment benefit in patient subsets. This paper provides detailed guidance for the evaluation of prognostic factors in cancer.

### *Adjustment for Covariates*

Randomization ensures that no systematic bias affects the treatment comparison, but it cannot ensure that the two treatment groups will be identical with regard to prognosis. It may happen by chance that more of the patients with a poor prognosis are assigned to one of the treatments. To prevent this type of imbalance from influencing the treatment comparison, one can perform an analysis that accounts for the effects of important prognostic factors, or covariates.

The most common method of adjustment is to perform a separate analysis for each level of the covariate (or for each possible combination of levels when multiple covariates are considered simultaneously) and statistically aggregate the results. For example, in a clinical trial of childhood acute lymphoblastic leukemia, one may want to adjust for age by considering outcomes separately for infants (<1 year), children between the ages of 1 and 10 years, and those older than 10 years because these groups have different prognoses. Even when no imbalances exist, this type of adjusted, or stratified, analysis is more efficient than an unadjusted analysis; by comparing outcomes within homogeneous subgroups, the variability of the overall result is slightly reduced. [156] Both adjusted and unadjusted analyses should be performed and reported whenever important prognostic factors have been identified for the patient population under study. A more complete discussion of the rationale and methodology for adjustment of comparative analyses can be found in the text by Friedman and colleagues [157] and in articles by Simon.[158] For studies intended to provide definitive evaluations, decisions about adjusting for particular covariates should be made before any data analysis to avoid the possibility that the selection of the adjustment strategy may have been influenced by the conclusions that would result. [159]

### *Meta-Analysis*

*Meta-analysis* is defined as a quantitative summary of research in a particular area. What differentiates a meta-analysis from the more familiar review of the literature is the construction of an overall summary result obtained by statistically aggregating the results of the reviewed studies. Formal meta-analysis, also referred to as an *overview*, is becoming more common in medical research and has been widely used in the social sciences for many years.

An extensive literature concerning the application of these techniques to clinical trial results is rapidly developing. [160,161,162,163,164,165,166,167,168,169,170] and [171] Many scientists believe that meta-analysis is proving to be a useful, perhaps even essential, tool in reaching reliable conclusions from the mixed assortment of studies that may have addressed the same research question.[172] Other scientists have expressed concerns about the validity of combining data from separate investigations and about whether the questions that can be properly addressed by these methods are of sufficient interest to warrant the effort they entail. [173] The Cochrane Collaboration, an international initiative to develop and maintain ongoing meta-analyses addressing important questions in medical research, has been highly influential in encouraging the performance of high-quality meta-analyses.[174]

A number of methodologic issues in the performance of meta-analyses have been raised. One concern is “publication bias”: whether meta-analyses that include only studies that have been published substantially overestimate treatment effects because negative studies are less likely to be published than positive studies. [175] Although publication bias is widely acknowledged to be a real concern, combining peer-reviewed reports with those that have not undergone peer review generates other concerns.[176] Another important issue centers on the “combinability” of studies. The question of which studies are sufficiently similar with regard to treatment, patient population, and methods of determining end points must be dealt with in planning any meta-analysis. Selection criteria should be determined in advance of reviewing potential studies: to avoid bias, it is optimal that studies be assessed for inclusion by reviewers who are blinded to the results of the study.

Meta-analysis of data from randomized trials will be unlikely to play a major role in pediatric cancer research because rarely do multiple studies compare similar regimens in the same disease population. The aggregation of nonrandomized studies may be more feasible in terms of patient numbers, but meta-analysis of nonrandomized studies is even more problematic because the selection biases that inevitably arise in uncontrolled studies may be multiplied if the results of such studies are formally combined.

## REPORTING RESULTS OF CLINICAL TRIALS

Ineffective reporting seriously compromises the value of well-conceived and expertly conducted clinical trials. These complex, time-consuming, and expensive investigations are conducted to increase the therapeutic information available to a medical community that relies on the published literature. Meaningful communication is therefore a critical component of the clinical trials process; without it, much of the investment in clinical trials is lost. Indeed, the publication of ambiguous or misleading results can be medically harmful and scientifically counterproductive.

The need for systematic investigation to identify advances in cancer treatment is widely accepted, and sophistication about issues of design and analysis in clinical trials has increased, but the methodology of reporting these trials has not always received the attention it merits. With the recognition that reliable comparisons among results of clinical trials can be made only in the presence of standardized reporting procedures, the World Health Organization in 1979 published recommendations for uniform approaches to assessment and reporting. These recommendations were developed and endorsed by an international assemblage of representatives of cancer clinical trials organizations. [87,177] Although these recommendations provide a useful starting point, they proved ineffective in circumventing the methodologic deficiencies increasingly observed in the clinical trials literature. [147,178,179,180] and [181] Various suggestions have been directed to medical journal contributors and editors in an effort to enhance the utility of reported results. [182,183,184,185] and [186] A useful development is the adoption by the editorial board of the *Journal of the National Cancer Institute* of a set of methodologic guidelines for reporting clinical trials. [187] These guidelines have been endorsed by editors of the *Journal of Clinical Oncology*[188] and *Cancer*.[189] Other articles providing useful guidance have been published by Zelen [190] and Bailar and Mosteller.[191] A checklist of information to be included in clinical trials reports, proposed by the Working Group on Recommendations for Reporting of Clinical Trials in the Biomedical Literature [192] has been superseded by the Consolidated Standards of Reporting Trials statement, [193] which advocates use of both a checklist and a flow diagram to assist editors and reviewers of randomized controlled trials. The Consolidated Standards of Reporting Trials approach has been adopted by *JAMA*[194] and *Lancet*.[195]

The value of any clinical trials publication is obviously related to the value of the experiment it reports. A useful publication is accurate, medically informative, and convincing to the reader. Accuracy is largely predetermined by the validity of the experimental design, the quality of its execution, and the legitimacy of its statistical analysis. The degree to which a paper is medically informative depends on the importance of the study question and the appropriateness of the experimental conditions. The ability to persuade the reader, however, relates largely to the information the researcher chooses to communicate. Although the general structure of

research papers (i.e., background, methods, results, and conclusions) is widely known, the detail presented is often insufficient to persuade a critical readership of the validity or applicability of the conclusions. Even when the design and conduct of the study and the analysis of results are impeccable, an inadequate description of these features prevents meaningful interpretation by the experienced audience for whom it is intended. This deficiency may delay the acceptance of an important advance or, more commonly, as in small studies with limited power, may suggest the acceptance or rejection of a concept based on what is an equivocal result that requires further investigation.[178]

To be maximally effective, the author of a clinical trials report should write from the perspective of a critical reader. In evaluating the manuscript, a reader initially wants to know the scientific motivation for the study, particularly the specific hypotheses addressed and the reasons for their plausibility and pertinence. The investigator must list all the hypotheses addressed in the study, not only the ones for which the results were statistically significant, to permit the reader to assess multiplicity issues. It may happen that the investigator has performed many comparisons, relating to secondary end points, strength of prognostic factors, treatment effect in subgroups, and so forth, but the report includes only those comparisons that result in a significant *p* value. The reader will interpret a report of a single positive test differently from a report of 40 tests with a single positive result.

A detailed methods section permits an assessment of the strengths of the design and provides an opportunity to replicate the effort, if warranted. This section should clearly describe the experimental conditions, including the specifics of patient registration procedures, inclusion and exclusion criteria, the target patient population, the details of the treatment regimen and any modifications, the schedule of follow-up evaluations, the procedures used to assess major end points (including whether the person making the end point evaluation was “blinded” to the treatment assignment), and, in comparative trials, the nature of the control group and the specific methods used for treatment assignment. The report should state whether the treatment allocation was by randomization. A description of the specific methods used to guarantee random treatment assignment and their timing relative to patient entry on study should be provided.[196] A brief description of quality control procedures can ensure the reader that the information reported is complete and accurate. Finally, a discussion of the statistical procedures used to analyze the data allows the reader to assess the reliability of the reported results. This description includes identification of analytic procedures used and explanatory material for techniques likely to be unfamiliar to the journal readership. References to articles or books describing all but the simplest and most standard techniques should be provided.

The results section presents the outcome of the experiment; clear and detailed exposition is crucial. A complete description of the patients entered on the study, including age, disease characteristics, nature and amount of prior therapy, and other items considered important in determining eligibility or establishing prognosis, should be reported. Toxicity and compliance information should be included, and outcomes for all patients entered should be reported. Confining information to patients deemed evaluable prevents accurate comparisons across studies whose policies regarding evaluability may differ.[187,190] If feasible, as with small studies, lists of individual end point determinations are useful.

Investigators must define end points carefully in reports of clinical trials. Common phrases such as “disease-free survival” are not necessarily defined in the same way by all investigators. The adoption of standard definitions and analyses, as proposed by Mastrangelo and colleagues[91] for pediatric leukemia studies and by the proponents of the Response Evaluation Criteria in Solid Tumors guidelines for solid tumors,[89] would facilitate the interpretation of study results and the comparison of results across studies.

Reports of clinical trials usually conclude with the author's interpretations of the study results. If the data have been analyzed appropriately, the conclusions are usually self-evident. Potential sources of bias, the need for independent confirmation, and any other warnings should be included in the discussion. Claims of patient benefit should be circumspect and based on the demonstrated difference in outcome between experimental and control groups, whose characteristics have been accurately described. Claims of no benefit should be accompanied by a confidence interval around the observed difference; a calculated probability (i.e., power) that a clinically important difference would have been detected with the sample size used may also be of interest.[197] Generalizations to a wider population should be made cautiously.

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# 19

# ACUTE LYMPHOBLASTIC LEUKEMIA

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## INTRODUCTION

The progress made in the treatment of acute lymphoblastic leukemia (ALL) of childhood is one of the true success stories of modern medicine ( Fig. 19-1). Incremental advances in treatment success span a 50-year period, during which ALL has gone from a uniformly fatal disease to one with an overall cure rate greater than 75%.[1,2,3,4 and 5] This extraordinary therapeutic progress is the result of treatment advances that began with the identification of effective single-agent chemotherapy in the late 1940s, followed by the development of combination chemotherapy and maintenance chemotherapy in the 1950s and early 1960s and the implementation of effective central nervous system (CNS) preventive therapy in the 1960s and 1970s. Continued, gradual improvement occurred through the 1990s. Additional information regarding cytogenetic, immunophenotypic, molecular characterization, and early treatment response help refine risk group categorization. This led to more appropriate “tailoring” of therapy, allowing a better defined “high” risk group to receive increasingly intensified therapy, whereas therapy for lower risk patients was successfully modified to reduce the risks of toxicity without compromising therapeutic efficacy. With the improving cure rate, the focus of future studies can move toward decreasing long-term side effects as well as examining short-term convenience and economic issues (i.e., outpatient versus inpatient delivery of the therapies). As the new century begins, the prospects for future progress fueled by advances in genomic research is bright.

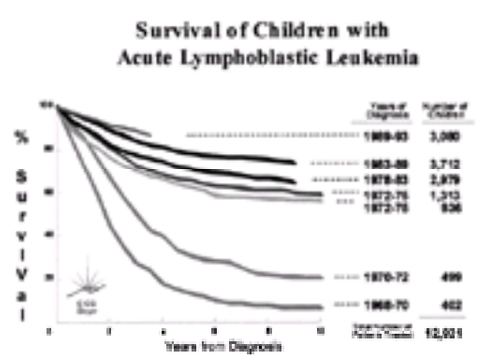


**FIGURE 19-1.** Improvement in survival of children with acute lymphoblastic leukemia. Curves represent survival outcomes for patients treated on successive Children's Cancer Group (CCG) clinical trials conducted over the 1968 to 1997 period. (A. Bleyer, H. Sather, *personal communication*, 2001.)

This chapter reviews the pathophysiology and biology of ALL, including new findings in the molecular biology of ALL, the applications of the new genome and molecular sciences in the initial diagnosis, minimal residual disease (MRD) detection, refinements in risk group stratification, and current treatment principles for ALL. Although the history of therapeutic approaches is briefly reviewed, the focus remains on current therapeutic strategies and on the unresolved treatment and etiology issues that represent the most significant current medical and scientific challenges.

## EPIDEMIOLOGY

Although the overall incidence of ALL has remained constant over the past three decades, new information concerning subgroups continues to be uncovered. [6,7 and 8] ALL is the most common malignancy in children. It accounts for one-fourth of all childhood cancers and approximately 75% of all cases of childhood leukemia. [5] Approximately 2,500 to 3,500 children are diagnosed with ALL each year in the United States, with an incidence of 3 to 4 cases per 100,000 white children. [9,10] The peak incidence of ALL occurs between age 2 and 5 years (Fig. 19-2) and may be trending downward in both the United States and Great Britain.[11,12] This young age peak historically has appeared at different times in different countries. It occurred initially in Great Britain in the 1920s, in the United States in the 1940s, and in Japan in the 1960s.[13] The appearance of these peaks corresponds to major periods of industrialization in these countries, suggesting that they may reflect different periods of exposure to new environmental leukemogens.[8,14]

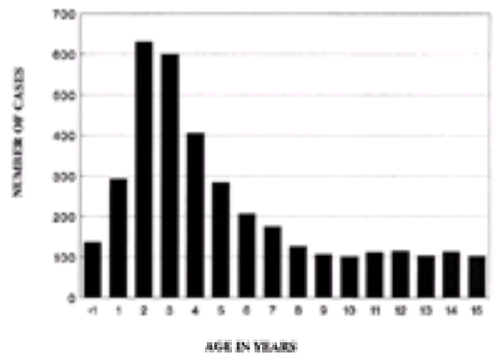


**FIGURE 19-2.** Age distribution of 3,620 children with acute lymphoblastic leukemia. (Data from the Children's Cancer Group, courtesy of H. Sather).

In the United States, ALL is more common among white children than black children. This may be due to the early surge in ALL incidence that occurs in whites. [3,15] This phenomenon may reflect a difference in susceptibility or in exposure to whatever environmental influences may be responsible for the early age peak in whites. Considerable controversy exists concerning whether outcomes differ between white and black children. [3,11,16] Reports from the 1960s and 1970s indicated that black children fared significantly worse compared with white children. [17] The observed differences had been postulated to be due to socioeconomic factors. In the late 1980s and early 1990s, several reports showed equivalent outcomes when the patients were carefully stratified according to risk group. [17] More recent studies, which account for most of the current significant prognostic variables, have shown statistically significant poorer outcomes for black compared to white children. These differences now appear to reflect the fact that a predominance of black patients fit into the higher risk categories, and that there may be specific pharmacogenetic differences between racial groups that result in disparities in outcomes.[3,16,18]

The incidence of ALL is higher among boys than girls, and this difference is greatest among pubertal children. [19] The male preponderance is particularly evident in cases of T-cell ALL.[20] Despite speculation about a possible role for sex hormones in leukemogenesis, none has been definitely established. One recent study in a population with relative genetic homogeneity (the French population of Quebec) demonstrated an increased risk for children with a particular set of glutathione S-transferase (GST) and cytochrome P-450 alleles.[21] A protective effect for girls was associated with the presence of a specific P-450 allele. [21] Other work has suggested an association between a certain HLA-DR haplotype (HLA-DRB4*01) and a particular mutation (C282Y) in the HFE gene (associated with hereditary hemochromatosis) and an increased risk for boys developing ALL.[22,23] These results need to be confirmed in studies of larger and genetically more heterogeneous populations. In addition, as the complexity of the genome is uncovered, it is likely that more complex environmental and genomic interactions are responsible for observed differences in ALL incidence.

It has been known for a long time that the general risk of ALL may be associated with specific environmental exposures (see the section Pathogenesis), as well as with specific but unknown enzymatic genotypes. As discussed later, polymorphisms of the GSTs and similar genes involved in the task of detoxification of drugs and xenobiotics have also recently been found to be important in determining response, side effects, and even the risk of relapse after completion of therapy. [24,25,26 and 27]

There also appear to be geographic differences in the frequency and age distribution of ALL. For example, ALL is relatively rare in North Africa and the Middle East, and non-Hodgkin's lymphoma is the most common childhood malignancy in those regions. In India and China, ALL is somewhat more common, but its incidence is still considerably lower than that in the industrialized West. [15] This geographic variation may reflect, in part, the distribution of different immunologic ALL subtypes. There appears to be a lower incidence of common ALL in developing countries and a higher incidence of T-cell ALL in the more industrialized countries. Although this may reflect an underdiagnosis of common ALL in developing countries, it is also possible that children in industrialized countries are more likely to be exposed to leukemogens that cause ALL.[28]

Several studies have suggested a link between maternal reproductive history and the risk of ALL. [29,30] Higher risk has been associated with a history of fetal loss. It is unclear whether this reflects genetic predisposition, an abnormal intrauterine environment, or a common environmental exposure. Increasing maternal age has been associated with an increased risk of ALL in the offspring. An association also has been posited between fetal loss and homozygosity at the same HLA-DR regions HLA-DRB4*01 (see above), which has been associated with increased ALL risk for males. [23] Higher birth weights appear to be associated with a greater risk of ALL, but the reason is unknown and may simply be related to the fact that birth weights tend to increase with maternal age. [29,31] Paternal chemical exposure (pesticides and fungicides) also has been associated with a higher incidence of childhood ALL. [32]

Numerous reports speak of the occurrence of leukemic clusters, which are observations of a greater than expected number of leukemia cases within a given period or geographic area (see Chapter 1). Under careful scrutiny, however, most purported clusters have not been substantiated. [15]

## GENETICS

Genetic factors are presumed to play a significant role in the cause of acute leukemias, including ALL (see Chapter 2 and Chapter 3). Evidence for this is based on several observations, including the association between various constitutional chromosomal abnormalities and childhood ALL ( Table 19-1), the occurrence of familial leukemia, and the molecular epidemiologic evidence of the importance of various alleles of specific genes (see above).

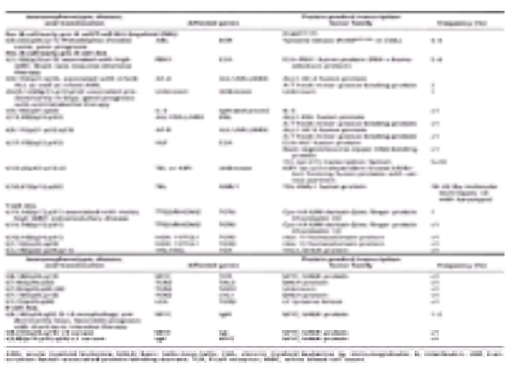

**TABLE 19-1. NONRANDOM CHROMOSOMAL TRANSLOCATIONS ASSOCIATED WITH SPECIFIC IMMUNOPHENOTYPES AND FUSION PROTEIN PRODUCTS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)**

Constitutional chromosomal abnormalities are associated with childhood leukemia. Children with trisomy 21 (i.e., Down syndrome) are up to 15 times more likely to develop leukemia than are normal children.[33] Although both ALL and acute myeloid leukemia (AML) are observed, ALL predominates in all but the neonatal age group.[34]

Other less common preexisting chromosomal abnormalities have been linked to leukemia.[35] Included among these are children with Klinefelter's syndrome and the trisomy G syndromes.[36] Children with neurofibromatosis and those with Schwachman syndrome are also reported to have an increased risk of leukemia.[37,38] As noted above, a higher risk of childhood leukemia has been associated with increasing maternal age. This may reflect the increased incidence of subtle karyotypic abnormalities in infants born to older mothers.[39]

The incidence of acute leukemia among those with Bloom syndrome and Fanconi's anemia is well documented.[40] These rare, autosomal recessive disorders are characterized by increased chromosomal fragility. Although AML is more common in those with Bloom syndrome, ALL also occurs.[41,42] There is some evidence that the development of leukemia in these patients is a consequence of genetic recombination of somatic cell chromosomes.[41] Defective replication and repair of DNA appears to play a significant role in both disorders.[43,44] Fanconi's anemia is most frequently associated with the development of acute myelomonocytic leukemia rather than with ALL.[45]

Lymphoid malignancies, with a predominance of T-ALL, have been reported in patients with ataxia-telangiectasia (AT), an autosomal recessive disorder characterized by increased chromosomal fragility.[46] B-cell and pre–B-cell ALL also have been reported occasionally in AT patients.[47] The gene responsible for AT (ATM) has been cloned, but the mechanism by which this increases patients' risk for cancer has not yet been uncovered.[48]

The importance of *in utero* genetic events has been suspected for many years due to concordance studies on twins with leukemia.[49,50 nd 51] The suggestion that leukemogenesis begins *in utero* also has been explored in studies that have found leukemogenic translocations [i.e., t(4;11) and t(12;21)] and markers of clonality that match that of the later leukemic blasts in heelstick blood samples (Guthrie cards) from newborns who later developed ALL.[52,53,54 and 55] Although these studies providing tantalizing clues that the process of developing leukemia may begin *in utero*, the questions of whether these genetic changes are the “accelerating,” permissive, or merely incidental genetic changes in patients who later developed leukemia remain unresolved. Crucial work remains to be done concerning whether the specificity of the leukemogenic mechanism will match the sensitivity of the polymerase chain reaction (PCR) methods used to test for small amounts of presumed leukemic cells. In the neonatal heelstick studies mentioned above, these patients were found to have 1/100 to 1/10,000 of the cells with DNA changes at birth, which contained one genetic alteration found months to years later in their leukemic blasts. It is not clear whether many more “normal” individuals (who never clinically present as leukemics) may show similar chromosomal breakpoints or evidence of clonal rearrangements in their white cells at birth.

Multiple cases of leukemia within families have been reported, including aggregates among siblings and groups within the same generation or in several generations.[56,57] The frequency of leukemia is higher than expected in families of leukemia patients.[58] Siblings of children with leukemia, including ALL, have an approximately twofold to fourfold greater risk of developing the disease than do unrelated children in the general population.[35,59] Although the occurrence of leukemia in identical twins has been used to support the role of genetic factors in the disease, the extent to which this association implicates a genetic susceptibility is ambiguous.[35] The concordance of acute leukemia in monozygotic twins is estimated to be as high as 25%. The risk for concordance among twins (both mono- and dizygotic) is highest in infancy; this risk diminishes with age, and after age 7 years, the risk to the unaffected twin is similar to that for persons within the general population.[35,57] Although the high concordance rate among younger twins suggests a genetic predisposition or *in utero* transfer of the leukemic cells, it may also be the result of simultaneous exposure to a common prenatal or postnatal leukemogenic event.

## PATHOGENESIS

In addition to genetics, environmental factors, viral infection, and immunodeficiency may predispose children to leukemia.

### Environmental Factors

Exposure to ionizing radiation and certain toxic chemicals can facilitate the development of acute leukemia. The high incidence of leukemia in survivors of the atomic bomb explosions in Japan during World War II is well documented.[60,61] The risk of leukemia was greatest for those closest to the explosion.[62] For persons who received exposure doses greater than 100 cGy, the dose-response relation for the production of leukemia was linear.[63] The type of leukemia observed was related to the age at exposure. ALL was seen more frequently in children, and AML was more common in adults.

Among survivors of the atomic bomb, there was no increase in the incidence of leukemia in children exposed to radiation *in utero*. This experience contrasts with other reports of an increased risk of leukemia in children exposed to diagnostic irradiation antenatally, particularly during the first trimester.[64] In a study by the National Academy of Sciences, a fivefold increased risk of all childhood cancers was found for children exposed to diagnostic radiation during the first trimester. When exposure occurred during the second and third trimesters, the risk was 1.5 times normal. Leukemias comprised approximately one-half of the cancers in that study; the increased risk for leukemia extended through age 12 years.[64] A significant leukemogenic effect has been reported in children exposed *in utero* to doses of 0.3 to 0.8 cGy.[65] Prenatal x-ray exposure, however, probably accounts for a very small portion of childhood ALL cases.[20]

The risk of developing leukemia from *ex utero* diagnostic irradiation is difficult to determine. One study suggested that approximately 1% of all cases of adult leukemia can be assumed to be a result of exposure to diagnostic radiography.[66] Therapeutic irradiation has been associated with a higher risk of acute leukemia in patients with ankylosing spondylitis treated with relatively high-dose radiation and in neonates administered thymic irradiation (which was once used to treat enlargement of the thymus).[67,68] An increased leukemic mortality rate was also observed in one study for children who received scalp irradiation for treatment of tinea capitis.[69]

Although the potential of ionizing radiation for causing leukemia is acknowledged, the actual percentage of leukemia cases directly attributable to radiation is presumed to be small. Controversy persists about the risks from exposure to ionizing radiation from routine emissions from nuclear power plants or as a result of fallout from atmospheric nuclear testing. Controversy also surrounds the possibility that exposure to electromagnetic fields (EMF) may be causally related to the development of childhood ALL. Conflicting studies exist in the literature. A case-control evaluation of a population of children in Denver suggested a twofold to threefold higher incidence of childhood cancers, including ALL, among children living in proximity to high-voltage power lines.[70] Similar studies from Sweden have shown increased risk from high-voltage power lines and from low-dose irradiation from ground current sources.[71,72] In contrast, studies of children living in Rhode Island and in Yorkshire showed no association between EMF and childhood ALL.[73,74] Later reviews, including large meta-analyses of multiple studies, present conflicting results.[75,76] Laboratory studies suggesting that EMF exposure might cause cancer by increasing cellular levels of the *myc* oncogene have been questioned by the failure of several groups to replicate the original work.[77,78] The most recent large case-control studies all have concluded that EMF exposure does not cause childhood ALL.[79,80]

Chronic chemical exposure (e.g., to benzene) has been associated with the development of AML in adults.[81] Direct evidence linking exposure to the development of childhood ALL does not exist. However, recent work with NAD(P)H:quinone oxidoreductase 1, one of the enzymes responsible for benzene and other quinone metabolism, has a mutation with decreased enzymatic activity that has been linked with the development of both AML and ALL in adults.[82] Patients with low NAD(P)H:quinone oxidoreductase 1 activity are less able to respond to oxidative stress, have evidence of increased numbers of chromosomal translocations, and have a general increase in the risk of developing leukemia. The GSTs represent another set of xenobiotic detoxifying enzymes with a known series of polymorphic mutations that effect function.[83] It has been suggested that GST null mutations may be associated with the development of infant and other subtypes of ALL.[84,85] There is substantial evidence that chemotherapy itself, particularly with alkylating agents, has leukemogenic potential. In a study of more than 9,000 2-year survivors of childhood cancer, a 14-fold excess of leukemia was observed, primarily attributable to therapy with alkylating agents.[86] Most of these cases, however, were AML. Other factors studied for possible association with ALL include parental cigarette smoking; herbicide and pesticide exposure; paternal military experience (particularly with service in Vietnam or Cambodia, in which servicemen were exposed to Agent Orange and other chemicals); maternal use of alcohol, contraceptives, and diethylstilbestrol; household radon exposure; and chemical contamination of ground water.[29,32,87,88,89,90 and 91] Definitive causal relationships between these factors and

childhood ALL have not been demonstrated.

### Viral Infection

There has been intense interest in the possible role played by viral infection in the pathogenesis of human leukemia. [92,93] and [94] This has been due in part to the fact that the young age of onset distribution of ALL corresponds with a time when the immune system is developing and is perhaps more vulnerable to the oncogenic effects of particular viruses. Some reports have suggested an increased risk for ALL in children born to mothers recently infected with influenza, varicella, or other viruses, but no definitive link between prenatal viral exposure and leukemic risk has been confirmed. No direct association between childhood or maternal viral infections and the occurrence of ALL has been documented, and those that have been investigated have not been shown to be clearly causative. [20,95] A possible inverse association with hepatitis A virus (as a measure of general hygiene) has been shown. [96] On the basis of this association, it has been hypothesized that the greater increase in ALL incidence in United States and Japanese populations compared to that in the less developed countries may simply be that children in the developed world are more "immunologically naive." Thus, they may be more susceptible to infectious/oncogenic agents acquired either *in utero* or early in life. Both the possible existence of a rare leukemogenic virus and the hypothesis that ALL represents a "rare response to a common infection" have stimulated continued investigation of a viral etiology. [94] Possible causal associations between human ALL and feline and bovine leukemia viruses and the polyomaviruses JC, BK, or simian virus 40 have never been confirmed.[95,98]

The Epstein-Barr virus (EBV) has been linked to cases of endemic Burkitt's lymphoma, the L3 morphologic subtype of ALL, and some cases of Hodgkin's disease. The EBV association is discussed further in the section on molecular genetics and in Chapter 24. The human lymphotropic viruses I and II are retroviruses that are implicated in some cases of adult T-cell and hairy cell leukemia. Cases of childhood malignancies have been linked to human immunodeficiency virus (HIV) infection, but the spectrum of histologies is different from those seen in adult acquired immunodeficiency syndrome patients. Pediatric acquired immunodeficiency syndrome patients have an increased incidence of non-Hodgkin's lymphoma (usually of B-cell origin; see Chapter 24), mucosa-associated lymphoid tumors (so-called MALT lymphomas), cystic tumors of the thymus, leiomyomas, leiomyosarcomas, and angiosarcomas.[99,100,101] and [102] Whether HIV or the immunodeficiency state that it induces is responsible for the increased rate of malignancies is unknown.

### Immunodeficiency

Children with various congenital immunodeficiency diseases, including Wiskott-Aldrich syndrome, congenital hypogammaglobulinemia, and AT (see the sections on epidemiology and genetics), have an increased risk of developing lymphoid malignancies, as do patients receiving chronic treatment with immunosuppressive drugs. These are usually lymphomas with mature B-cell phenotypes. Although ALL may occur in these circumstances, it is uncommon. Individuals with AT and Fanconi's anemia have increased chromosomal fragility and frequent abnormalities of chromosomes 14 and 7, suggesting that genetic mechanisms are important in these disorders.[103]

Abnormalities of the immune system are occasionally observed in newly diagnosed patients with ALL. [104] Abnormally low serum immunoglobulin levels have been observed in as many as 30% of these patients. Whether such abnormalities precede the development of leukemia or are a consequence of the disease is unclear. Similarly, abnormalities of the immune system may persist after therapy, although the effects of therapy versus those of the leukemia may be difficult to discern. [105] In addition to possibly contributing to the etiology of ALL, altered immune status may effect susceptibility to relapse or the development of second malignancies after completion of therapy (see the section Late Effects of Treatment; see also Chapter 14 and Chapter 17).

### Clonal Pathogenesis

ALL, similar to other lymphoid malignancies, is believed to develop as a consequence of malignant transformation of a single abnormal progenitor cell that has the capability to expand (into a so-called clone of similar progeny cells) by indefinite self-renewal. It is not entirely clear where in the normal course of differentiation the leukemic "clonal event" occurs, and it may actually be highly variable. In pediatric ALL there is evidence that these events occur in committed lymphoid precursors, whereas in AML and Philadelphia chromosome–positive ($Ph^+$; see the section Cytogenetics) ALL, it appears that they may occur earlier because there is evidence of mutation in multiple cell lineages. [106,107] The events that lead to the process of malignant transformation are complex and multifactorial. It has been proposed that ALL results from spontaneous mutation(s), which may occur in lymphoid cells of B- or T-cell lineage or in their precursor cell(s). [54,108] As noted previously, there is emerging evidence that the causative mutations may occur years before the presence of clinical leukemia. During normal lymphoid development, lymphocyte precursors may be at higher risk for spontaneous mutation because of the intrinsic, regulated, mutagenic activity occurring during the process of gene rearrangement and the high rate of proliferation in these cells. Many of the described molecular mutations bear evidence of immunoglobulin VDJ and T-cell receptor (TCR) recombinase activity. [109]

Greaves[108] theorized that one or, more likely, two sequential mutations spontaneously occurring in important regulatory genes in a lymphoid cell population undergoing significant proliferative stress could account for most ALL cases. Elaborating on this hypothesis, Greaves [108] suggested a model, analogous to that proposed in Knudson's two-hit hypothesis for the origin of embryonal malignancy, in which two distinct genetic events, one initiational and the other promotional, are involved in leukemogenesis. This hypothesis, which is a particularly attractive explanation for the development of B-cell precursor ALL, requires further confirmation. The recent *in utero* mutational findings discussed above may indeed localize the timing (if not the mechanism) of the initiational events.

Other support for the clonal expansion theory comes from classic studies of glucose-6-phosphate dehydrogenase isotypes, and cytogenetic and molecular studies. [110] Rearrangement of immunoglobulin and TCR genes also has been studied as a marker of clonality in ALL of pre-B lineage. [109,111] In most cases, identical patterns of immunoglobulin and TCR gene rearrangement are observed in leukemic cells obtained at diagnosis and relapse. [107,111,112] Infrequently, clonal variations occur in serial samples, suggesting polyclonal disease, or clonal progression. In most of these cases, however, the leukemia cells share at least one identical immunoglobulin gene rearrangement, implying a common clonal origin.[112]

For many years, it has been assumed that cure of ALL implied the killing of all leukemic cells. This may not be true, because multiple MRD technologies (see the section Minimal Residual Disease) are occasionally reported to show evidence of viable leukemic cells late and even off-therapy in patients who do not clinically relapse. Whether these cells truly represent clonal cells identical to those at leukemic diagnosis (versus a vestige of a preleukemic clone) is the subject of intense scrutiny. Another hypothesis would accept these cells as being those of the original clone; however, the patient's own immune system may have learned to control their proliferation.[111]

### Molecular Pathogenesis

The identification of chromosomal translocations in leukemic blasts led to the eventual identification and cloning of the individual genes disrupted by these events (see the section Cytogenetics and Table 19-1). Many of these cytogenetic changes occur at the location of immunoglobulin, TCR, and various transcription factor genes. In addition to chromosomal translocations, there are a variety of genetic events that appear to be leukemogenic but are undetectable with classic cytogenetic methods. These include small deletions, mutations, or chemical alteration (i.e., methylation) of DNA that can inactivate tumor suppressor genes or activate oncogenes. Point mutations can result in missense, nonsense, or frame shift mutations. These molecular lesions are discussed in greater detail in Chapter 4.

DNA mutations or alterations in the protein expression of p53, MDM2, p16, or p15, the interferon genes located on 9p, WT1 (i.e., the Wilms' tumor gene located at 11p13), the TEL and KIP1 loci on 12p12-p13, and others have all been described in fresh ALL samples and ALL-derived cell lines. [113,114,115,116,117,118] and [119] Although the abnormalities detected in most of these genes may not be as definitively leukemogenic as the bcr-abl and other fusion gene mutations described later (Table 19-1), they are commonly observed in pediatric ALL and appear to contribute both to the development of leukemia and (in some cases) to the response to specific chemotherapeutic agents.[120]

p53 is the gene most frequently found to be altered in human cancers (see also the discussion of p53 in Chapter 3).[121] Studies of p53 sequence, structure, and function in pediatric ALL have revealed a low rate of the same types of missense mutations and loss of heterozygosity of the wild-type allele as that found in solid tumors.[114,122,123] These changes are associated with the production of a mutated p53 protein. A number of cases of pediatric ALL have had deletions and large chromosomal rearrangements that resulted in the total loss of p53 protein. [114] The p53 mutations are predominantly found in T-cell leukemias. [124,125] and [126] Because p53 abnormalities are more commonly observed at relapse and occur at a relatively high rate (greater than 60%) in T-cell lines derived from relapsed patients, some investigators believe that they may not be directly causative in these leukemias. Alternatively, it has been hypothesized that p53 mutations may function as progression factors involved in the biologic events that lead to relapse or disease that is refractory to treatment. [120] In support of this hypothesis, several studies have

shown p53 mutations or overexpression of MDM2 (a protein that binds and causes p53 inactivation) in the blasts of patients who were refractory to treatment or had early relapse.[113,127] The exact role of p53 mutations in different types of ALL is unknown. Mutations have been identified in cases involving virtually every level of ALL differentiation.[128]

Another potential mechanism in the development of ALL involves mutational events that prevent apoptosis (programmed cell death). Originally cloned from the oncologic translocation breakpoint, t(14;18)(q21;q32), common in adult follicular and diffuse B-cell lymphomas, the Bcl-2 protein is able to prevent apoptosis and immortalize cells in tissue culture and transgenic murine models.[129,130] In transgenic murine models it not only prevents the normal apoptosis seen in developing lymphoid cells but also causes an expanded lymphoid compartment and leads to a high rate of lymphoid malignancies.[131] Several groups have noticed that increased Bcl-2 levels in leukemic blasts correlate both with their ability to grow in tissue culture and with poorer prognosis in ALL and AML.[131,132] and[133] However, there is an innate redundancy in the apoptotic pathways, such that apoptosis mediated by Fas or even wild-type p53 may be able to overcome a lack of Bcl-2 in some ALL blasts.[134]

## PATHOBIOLOGY

Morphologic, immunologic, cytogenetic, biochemical, and molecular genetic characterizations of leukemic lymphoblasts have confirmed that ALL is a biologically heterogeneous disorder. This heterogeneity reflects the fact that the leukemia may develop at any point during the multiple stages of normal lymphoid differentiation.

### Morphologic Classification

There have been several attempts to classify ALL cells morphologically using criteria such as cell size, nuclear to cytoplasmic ratio, nuclear shape, number and prominence of nucleoli, nature and intensity of cytoplasmic staining with a variety of staining agents, presence of cytoplasmic granules, prominence of cytoplasmic vacuoles, and the character of nuclear chromatin (Table 19-2 and Table 19-3).[135,136] Most of these efforts were unsuccessful because they were technically difficult to reproduce or lacked meaningful clinical correlations.[137,138] One system, however, proposed by the French-American-British (FAB) Cooperative Working Group has become generally accepted.[139,140] The FAB system (Fig. 19-3 and Table 19-2) defines three categories of lymphoblasts. L1 lymphoblasts are usually smaller, with scant cytoplasm and inconspicuous nucleoli. Cells of the L2 variety are larger, and they demonstrate considerable heterogeneity in size, prominent nucleoli, and more abundant cytoplasm. Lymphoblasts of the L3 type, notable for their deep cytoplasmic basophilia, are large, frequently display prominent cytoplasmic vacuolation, and are morphologically identical to Burkitt's lymphoma cells.

| Cytologic Features | L1 | L2 | L3 |
|---|---|---|---|
| Cell size | Small cells predominate | Large, heterogeneous in size | Large homogeneous |
| Nuclear chromatin | Homogeneous in very one case | Variable; heterogeneous in any one case | Finely stippled and homogeneous |
| Nuclear shape | Not visible or small and inconspicuous; none vesicular | Irregular clefting and indentation common | Regular—oval to round |
| Nucleoli | Regular; occasional clefting or indenting | One or more present; often large | Prominent; one or more |
| Amount of cytoplasm | Scanty | Variable; often moderately abundant | Moderately abundant |
| Basophilia of cytoplasm | Slight or moderate; rarely intense | Variable; deep in some | Very deep |
| Cytoplasmic vacuolation | Variable | Variable | Often prominent |

**TABLE 19-2. FRENCH-AMERICAN-BRITISH CLASSIFICATION OF LYMPHOBLASTIC LEUKEMIA**

| | Acute lymphoblastic leukemia | Acute myeloid leukemia |
|---|---|---|
| **Characteristic** | | |
| Nuclear/cytoplasmic ratio | High | Low |
| Nuclear chromatin | Clumped | Spongy |
| Nucleoli | 0–2 | 2–5 |
| Granules | – | + |
| Auer rods | – | + |
| Cytoplasm | Blue | Blue-gray |
| **Cytochemical reaction** | | |
| Peroxidase | – | + |
| Sudan black B | – | + |
| Periodic acid–Schiff | + | – |
| Naphthyl ASD chloracetate esterase | – | + |
| α-Naphthyl acetate esterase | – | + |
| α-Naphthyl butyrate esterase | – | – |
| Terminal deoxynucleotidyl transferase | +* | – |

+, cytochemical stain positive; +/–, cytochemical stain equivocal; –, cytochemical stain negative.
Note: Table provides information on characteristics that may be useful in differentiating acute lymphoblastic leukemia from acute myeloid leukemia. Wide variation in morphology is encountered in both disease categories. Diagnostic evaluation should use other studies, including immunophenotyping and cytogenetics.
*Terminal deoxynucleotidyl transferase is usually negative in typical French-American-British L3 acute lymphoblastic leukemia.

**TABLE 19-3. MORPHOLOGIC, CYTOCHEMICAL, AND BIOCHEMICAL CHARACTERISTICS HELPFUL IN DIFFERENTIATING ACUTE LYMPHOBLASTIC LEUKEMIA FROM ACUTE MYELOID LEUKEMIA**

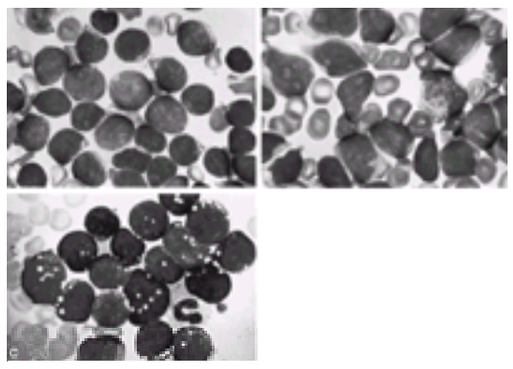

**FIGURE 19-3.** Morphologic appearance of acute lymphoblastic leukemia cells classified according to the French-American-British system. **A:** L1 morphology. **B:** L2 morphology. **C:** L3 morphology.

Approximately 85% of children with ALL have predominant L1 morphology, 14% have L2, and 1% have L3.[141] The L2 subtype is more common in adults.[142] Lymphoblasts of the L3 type possess cell surface immunoglobulin and other characteristic B-cell markers. There is, however, no apparent correlation between the FAB L1 and L2 morphologic types and immunologic cell surface markers.[143,144] Concordance among investigators using the FAB system is relatively high.[141,145,146] Since its original description, refinements of the FAB system have been proposed.[147,148] Although the existence of different approaches to FAB classification can confound interstudy comparisons, a variety of individual studies have demonstrated that the FAB classification has prognostic value.[147,149,150]

L1 morphology has been associated with a higher remission induction rate and better event-free survival (EFS) than L2 morphology, which appears to convey poor prognosis.[147,150] In early studies, L2 morphology appeared to be an independent prognostic variable indicative of poor outcome.[148] In more recent studies, however, it sometimes loses its predictive value when the patients are stratified for age, sex, and diagnostic white count.[136] Patients with the L3 morphology have the worst overall prognosis.[151,152] Although the FAB classification system appears to have value as a prognostic indicator, no biologic basis for the morphologic differences

delineated by this system has been identified. Generally, the most important morphologic distinctions are those between ALL and AML ( Table 19-3). Routine Wright's and cytochemical stains are usually adequate for this task, but fluorescent antibody cell sorting (FACS) and chromosomal analysis can be helpful in equivocal cases.

An unusual morphologic variant of ALL is the so-called hand mirror–cell variant in which leukemic cells are characterized by a hand mirror shape caused by a handle-shaped uropod.[153,154] Approximately, 5% to 23% of pediatric ALL cases are said to have this morphology.[136,155] The data concerning the prognostic implications of hand mirror morphology have been mixed.[136,155,156] The suggestion that the hand mirror–cell variant is associated with the development of CNS disease has not been confirmed.[157,158] In adult ALL the hand mirror morphology has been associated with a subset of female patients whose leukemic blasts display myeloid and lymphoid antigens (i.e., mixed phenotype) and whose clinical course is relatively indolent despite the fact that they rarely enter complete remission. [159,160]

Cytochemical stains have been studied with respect to their ability to differentiate between various clinical and immunologic subsets of ALL. The periodic acid–Schiff, acid phosphatase, b-glucuronidase, and acid a-naphthyl acetate esterase reactions have been evaluated. [161,162,163,164] and [165] Although some correlations appear strong (e.g., strong focal paranuclear acid phosphatase activity appears to be more common in T-cell disease), the practical use of this type of information is limited and has been supplanted by more sophisticated immunologic techniques, such as immunophenotyping with FACS. In rare cases of acute leukemia that cannot be definitively classified by current immunologic or molecular methods, ultrastructural detection of platelet peroxidase or myeloperoxidase may be helpful in identifying the megakaryocytic or myeloid nature of the disease.[166]

**Immunobiology**

Studies of the immunobiology of ALL have confirmed that leukemic transformation and clonal expansion can occur at different stages of maturation in the process of lymphoid differentiation (Fig. 19-4 and Fig. 19-5). In the early 1970s, when the first surface markers were used to characterize ALL in terms of cell origin and stage of differentiation, three immunologic subsets were delineated: T-cells, B-cells, and non–T/non–B-cells. Using receptors for sheep erythrocytes, approximately 20% of pediatric patients with ALL were found to have T lymphoblasts. [167] Cell surface immunoglobulin and complement receptors identified ALL of B-cell origin in 1% to 2% of patients. With these older immunologic methods of characterization, the remaining patients with ALL had no detectable cell surface markers on their blasts and thus were considered to have non-T, non–B-cell or so-called null cell leukemia. [168] The development of heterologous antisera and monoclonal antibodies directed against human leukemia–associated antigens indicated that approximately 80% of patients formerly presumed to have had non-T, non–B-cell ALL had a common ALL antigen (CALLA), CD10, on their cell surface.[169,170] and [171] This leukemic subset is now referred to as *CALLA+*, *CD10+*, or *common ALL*.

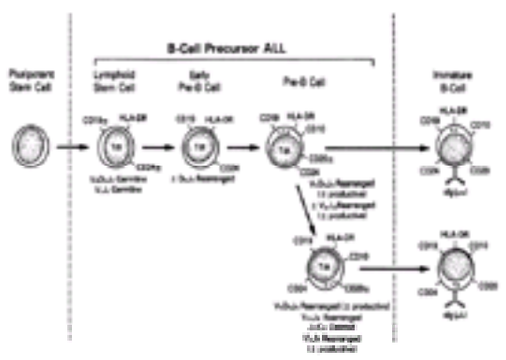


**FIGURE 19-4.** Schematic representation of stages of lymphoid differentiation found in B-cell precursor acute lymphoblastic leukemia (ALL) of childhood. Stages are delineated on the basis of reactivity with commonly used monoclonal antibodies, immunoglobulin and T-cell receptor gene rearrangement, terminal deoxynucleotidyl transferase activity, and presence or absence of cytoplasmic or cell surface immunoglobulins. (From Felix CA, Poplack DG. Characterization of acute lymphoblastic leukemia of childhood by immunoglobulin and T-cell receptor gene patterns. Leukemia 1991;5:1015–1025, with permission.)

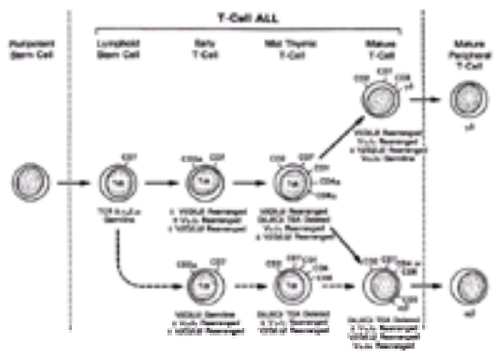


**FIGURE 19-5.** Schematic representation of stages of lymphoid differentiation found in T-cell acute lymphoblastic leukemia (ALL) of childhood. Stages are delineated on the basis of reactivity with commonly used monoclonal antibodies, immunoglobulin and T-cell receptor gene rearrangement, and terminal deoxynucleotidyl transferase activity. (From Felix CA, Poplack DG. Characterization of acute lymphoblastic leukemia of childhood by immunoglobulin and T-cell receptor gene patterns. Leukemia 1991;5:1015–1025, with permission.)

Most leukemias previously determined to be of the non-T, non-B type are actually of early B-cell lineage. The demonstration of intracytoplasmic immunoglobulin in some of these cells, their reactivity with monoclonal antibodies specific for B-cell associated antigens, and their ability to differentiate *in vitro* into cells with mature B-cell markers confirmed that approximately 80% to 85% of childhood ALL cases develop as a result of the monoclonal proliferation of B-cell precursors. [172,173] The presence of cytoplasmic immunoglobulin (cIg) has been a useful marker to determine the level of differentiation of leukemic cells of B-cell lineage. [146,174] cIg exists in approximately 20% to 30% of cases of B-cell precursor ALL.

The use of monoclonal antibodies, improvements in the enzymatic and fluorescent tagging of these antibodies, and the development of the multiparameter FACS machine have revolutionized pathologic classifications of many diseases, including ALL. [175] More than 200 different monoclonal antibodies are commercially available that can detect antigens associated with the different hematopoietic lineages. Those most helpful in the immunologic classification of ALL are shown in Table 19-4. Using a panel of monoclonal antibodies associated with various stages of B-cell differentiation along with information on the presence or absence of cytoplasmic and surface immunoglobulin, investigators have classified B-lineage ALL into discrete stages according to the degree of differentiation or maturation ( Fig. 19-4).[141,143,176] However, none of the monoclonal antibodies used in routine clinical immunophenotyping is absolutely lineage specific. [177,178] *Lineage associated* is the preferred terminology. To accurately immunophenotype most cases requires use of a panel of multiple antibodies. The choice of the diagnostic panel may vary among institutions and laboratories, but for the characterization of lymphoid leukemias antibodies for several T-cell antigens (i.e., CD3, 5, or 7) and early-B lineage, antigens CD10, 19, and 22 are generally used.

| CD* | Predominant reactivity |
|---|---|
| **T Cell** | |
| CD1 | Thymocytes |
| CD2 | Pan-T |
| CD3 | Pan-T |
| CD4 | T helper/inducer |
| CD5 | Pan-T, B-CLL |
| CD7 | Pan-T |
| CD8 | T cytotoxic/suppressor |
| UCHT1 | T81/3B9 = major subset |
| | T4/OKT4+ = suppressor inducer |
| **B Cell** | |
| CD19 | Pan-B |
| CD20 | Pan-B |
| CD21 | C3dR |
| CD24 | Pan-B |
| | Plasma cells |
| **Myeloid** | |
| CD11c | Monocyte, hairy cell |
| CD13 | Pan-myeloid |
| CD14 | Monocytes |
| CD15 | Monocytes, granulocytes |
| 1B23 | Pan-myeloid |
| **Miscellaneous** | |
| CD9 | Hematopoietic |
| | Progenitor/leukemic blasts |
| CD10 | ALL/lymph w/follicular |
| | lymphoma |
| CD34 | Hematopoietic gemtor cells/ human lymphotro- |
| | pic virus–infected cells |
| CD41a | Platelets/megakaryocytes |
| CD45 | Pan-leukocyte |
| | Transferrin receptor/proliferating cells |

ALL, acute lymphoblastic leukemia; B-CLL, B-cell chronic lymphocytic leukemia.

*The clusters of monoclonal antibodies and their predominant reactivity are listed.

From Poplack DG: Clinical manifestations of acute lymphoblastic leukemia. In: Hoffman R, Shattil S, Furie B, et al., eds. Hematology: basic principles and practice. 2nd ed. New York: Churchill Livingstone, 1991:716, with permission.

**TABLE 19-4. MONOCLONAL ANTIBODIES COMMONLY USED TO IMMUNOPHENOTYPE LEUKEMIA**

There are some prognostic differences between the various precursor B-lineage ALL subgroups. Mature B-cell ALL has a poorer prognosis than earlier B-lineage subgroups. The distinction between patients with pre–B-cell (cIg+) ALL and those with early pre-B ALL (cIg–), however, does not seem to be prognostically relevant as long as the patients are stratified by risk group criteria and treated accordingly (see the section Treatment).[5,146,179] Patients with B-cell precursor ALL whose lymphoblasts manifest CALLA (CD10) have a more favorable prognosis.[145,146] Expression of the stem cell antigen CD34, present on approximately two-thirds of B-cell precursor ALL, also appears to be associated with a good prognosis.[180,181 and 182]

Identification of immunoglobulin gene rearrangement is helpful in confirming the B-cell precursor lineage of ALL cells otherwise devoid of other B-cell or pre–B-cell markers.[145,146] There is a hierarchy of immunoglobulin gene rearrangements in B-cell precursor ALL that mirrors different stages of normal B-cell differentiation ( Fig. 19-4).[146,183] Heavy-chain rearrangement precedes k light-chain rearrangement, which precedes l light-chain rearrangement. [143,183,184 and 185] As shown in Figure 19-4, it is possible to relate the pattern of immunoglobulin gene rearrangement to the discrete stages of lymphoid differentiation.

Although many cases fit the hypothesis that ALL is a disorder characterized by the clonal expansion of cells representing a specific stage of normal differentiation, it is evident that many B-cell–lineage leukemias exhibit a differentiation antigen pattern or immunoglobulin gene rearrangement profile that is not synchronous with any of the normal stages of differentiation.[186,187] In addition to this asynchrony of antigen expression, leukemic cells from some patients manifest characteristics of more than one lineage. The significance of such “lineage infidelity” in the display of immunophenotypic markers is controversial. [177,188]

Heavy-chain immunoglobin gene rearrangement has been observed in approximately 10% to 15% of T-cell ALL cases. [176,189,190 and 191] This phenomenon of “lineage spillover” indicates that heavy-chain rearrangement alone is an insufficient basis for assigning B-cell lineage. [190,192] TCR gene rearrangement also occurs with relatively high frequency in B-cell precursor ALL.[192,193] B-cell precursor ALL devoid of immunoglobulin (or TCR) rearrangement has been described. This germline configuration appears to be a characteristic of some cases of B-cell precursor ALL of infancy. [190]

T-cell ALL has distinctive immunobiologic as well as clinical features (see the section on prognostic factors). Molecular genetic analysis of the genes encoding the TCR provides a useful molecular marker of T-cell lineage commitment and the stage of T-cell differentiation. Study of the d, g, b, and a TCR gene rearrangement in T-cell ALL reveals a hierarchy of sequential TCR activation events that can be roughly correlated with the sequence of T-cell surface antigen expression in a fashion analogous to the hierarchy of immunoglobulin gene rearrangement found in B-cell precursor ALL ( Fig. 19-5).[176,194] Although molecular genotyping can provide insight into the developmental state of T-cell ALL, use of molecular markers alone to designate T- or B-cell lineage is inappropriate. As discussed above, immunoglobulin gene rearrangements may occur in T-cell ALL, and TCR gene rearrangements are observed even more frequently in B-cell precursor ALL. [176,195] Such lineage spillover may represent leukemias derived from cells at an early stage of lymphoid development, when TCR and immunoglobulin genes are accessible to a common recombinase enzyme. Cases of T-cell ALL putatively derived from the earliest stages of differentiation have neither immunoglobulin nor TCR rearrangements.

T-cell ALL has been subclassified using monoclonal antibodies, which detect surface antigens present at discrete stages in the process of normal T-cell differentiation or maturation.[168,196] Three stages of normal intrathymic differentiation have been proposed: early (stage I), intermediate (stage II), and late (stage III; Fig. 19-5). T-cell maturation involves a continuum of phenotypic changes, and T cells can be identified that are presumably derived from each of these different stages of differentiation.[168] Most T-cell leukemias display the antigen pattern of the early thymocyte stage I. In contrast, malignant cells from patients with T-cell lymphoma generally manifest an intermediate or a mature phenotype.[168] As in B-cell precursor ALL, however, numerous reports exist of T-cell ALL cases expressing immunophenotypes not typically representative of the normal stages of T-cell maturation. [186]

As noted previously, not all ALL cases adhere to a specific lineage. Comparisons between normal and leukemic cells using monoclonal antibodies or molecular genotyping have verified that numerous cases occur in which the leukemic cells express characteristics of more than one hematopoietic lineage. [197,198 and 199] In biphenotypic or acute mixed-lineage leukemia, lymphoid and myeloid characteristics are present on the same leukemia cell. Bilineal or biclonal leukemias are those in which there are two distinct populations of cells, one lymphoid and the other myeloid. *Lineage switch* (or *lineage shift*) is the term used to describe a conversion from one phenotype at diagnosis to a different phenotype at relapse.

Confirmation of the existence of mixed-lineage leukemia usually requires the use of immunophenotypic, molecular, karyotypic, and cytochemical information. When initial reports of mixed-lineage leukemias first appeared, it was assumed that dual markers represented an artifact produced by phenotypic markers that lacked specificity. However, numerous well-documented cases exist that meet even the most stringent classification criteria. It is apparent that the simultaneous expression of lymphoid and myeloid markers occurs more commonly than previously believed. In various series of pediatric ALL cases, the incidence of myeloid marker expression has ranged from 7% to 25%.[188,200,201 and 202]

The biologic basis for the appearance of mixed-lineage leukemias is not understood. It has been suggested that they occur as a result of inappropriate or aberrant gene activation and thus do not represent leukemias derived from a corresponding normal stage of hematopoietic development. [198] Alternatively, it has been proposed that mixed-lineage leukemias represent the clonal expansion of normal, bilineage, or multilineage potential precursors, which are difficult to detect in normal bone marrow.[198] Mixed-lineage leukemia is sometimes associated with particular cytogenetic and molecular findings; there is a higher incidence of mixed-lineage phenotype seen in the abnormal 11q23 cases, especially in the t(4;11)(q23;q23) cases, and in leukemias containing Ph, t(9;22). [5,203,204] The 11q23 abnormalities and the t(9;22) cytogenetic abnormalities are independently associated with poor prognosis (see the section on cytogenetics). It is unclear whether expression of one or more myeloid antigens in a subset of these lymphoid leukemias conveys further prognostic significance.

Although in the past, controversy existed over the preferred treatment of mixed-lineage leukemias, most investigators now agree that with modern chemotherapy regimens, there is no difference in outcomes between myeloid antigen–postive and myeloid antigen–negative ALL. Thus therapy should not be altered for this finding.[188,200,201 and 202] Confounding features in these studies have been differences in the actual treatment regimens used, differing definitions of myeloid antigen positivity, and occasionally differences in the source of the antibodies and methodologies used to phenotype the cells. [178]

In addition to immunophenotyping, which is essentially a gene expression study, other gene expression modalities are beginning to be used to characterize leukemias. These include complementary DNA (cDNA) microarray (Fig. 19-6A), real-time and other modifications of reverse transcriptase-PCR (RT-PCR), which examine messenger RNA expression levels, and new mass spectroscopy techniques for proteins (proteomics). [205,206 and 207] These and other new techniques allow researchers to examine large patterns of gene expression at either the RNA or protein level. If specific patterns can be correlated with clinical response, these new characterization methods should allow increased refinement of current prognosis-based stratification systems.

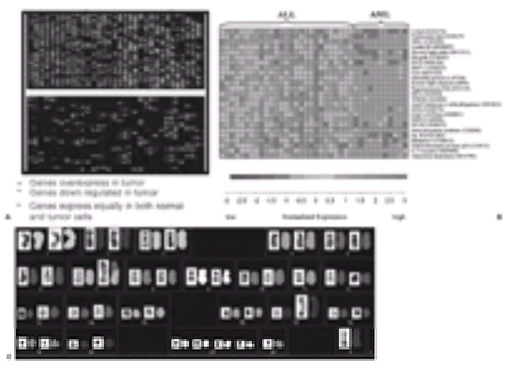

**FIGURE 19-6.** Application of new techniques for the molecular and cytogenetic characterization of acute lymphoblastic leukemia (ALL) blasts. **A:** Complementary DNA/messenger RNA (cDNA/mRNA) microarray. There are several current versions of this technology, including spotted cDNA arrays and oligonucleotide arrays. This example is of a cDNA array undergoing competitive hybridization from two sources of fluorescent-labeled RNA. The relative expression of each of 6,000 genes is measured by quantifying the amount of light emitted at the designated wavelengths at each spot on the array. **B:** Clustering of expression data on 11,000 genes (on an Affymetrix oligonucleotide array) from 38 cases of leukemia, showing that it is possible to use this technology to see differences as well as similarities among disease cases. (From Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science 1999;286:531–537, with permission.) **C:** Spectral karyotyping (SKY) on leukemic ALL blast cells. This case is remarkable for aneusomy (abnormal number) of chromosomes 21 and 22 as well as several complex marker chromosomes [45, der(X)t(X;17)(p21;q24), –Y, der(7)t(5;7)(a12;p22), and ider(17)(q10)t(X;17)(?q11)]. AML, acute myelogenous leukemia; IL-7, interleukin-7. (SKY results courtesy of X.Y. Lu, C.P. Harris, C.C. Lau, and P.H. Rao, *personal communication*, 2001.) (See Color Figure 19-6.)

Although immunophenotyping and cytogenetic analysis arise from different scientific disciplines, it has now become apparent that specific combinations of antigen expression correlate with the likelihood of detecting specific molecular genetic changes. [208,209,210 and 211] Advances in immunophenotyping reagents and instrumentation are also opening up new avenues of leukemic clone characterization—that is, it may be possible to quantitatively compare and find prognostic significance in the fluorescence intensity of specific antigens. [212] Multicolor/multiparameter FACS, which now permits the detection of as many as five different antigens simultaneously expressed on the same cell, is sensitive enough to determine the clonality of blast populations as well as detect phenotypically abnormal cells with a sensitivity of $10^{-4}$.[111] Current research is evaluating to what extent these newer FACS techniques may provide for accurate correlation with molecular cytogenetic changes and clinical outcomes.

## CYTOGENETICS

The improvement in the technology of cytogenetic analysis has increasingly contributed to the understanding of the biology and treatment of ALL. When combining the newer methods of chromosomal banding and standard fluorescent *in situ* hybridization (FISH) with the molecular genetic techniques of spectral karyotyping (SKY) (Fig. 19-6B) and comparative genomic hybridization (CGH), abnormalities can be recognized in the leukemia cells of virtually 100% of cases of pediatric ALL.[213,214,215,216,217 and 218] The SKY technique uses 24 color chromosomal paints (one specific to each chromosome). These paints allow reliable, genome-wide assignment of the chromosome origin of material in complex translocations that are often below the level of standard Giemsa detection. CGH is particularly suited for detecting losses or gains of material (e.g., deletions, duplications, and amplifications) that can be missed by both SKY and standard cytogenetic techniques. CGH also has the advantage of not requiring metaphase cells or cell culture of the diagnostic material. Both SKY and CGH are currently research techniques, which are used in conjunction with standard cytogenetics. These new methods require specific computer and fluorescent microscopy workstations and a licensed clinical cytogeneticist for proper clinical interpretation and correlation with the diagnostic Giemsa banded karyotype.

The cytogenetic abnormalities reported in ALL involve both chromosomal number (ploidy) and structural rearrangement. [219,220,221 and 222] The following sections discuss the molecular and clinical correlates of specific chromosomal changes in ALL. Their implications for treatment are discussed in the section on treatment.

### Ploidy

Ploidy can be determined directly by the classic method of counting the modal number of chromosomes in a metaphase karyotype preparation, or by an alternative indirect method of measuring DNA content by flow cytometry.[221] The DNA content by flow cytometry is measured in a DNA index (DI), which is a ratio between the normal amount of fluorescence seen in a diploid cell and the fluorescent content of the bone marrow blasts (in $G_0/G_1$) at diagnosis.[223] Normal diploid or pseudodiploid cells (cytogenetically abnormal but have a normal DNA content) have a DI of 1.0. Hyperdiploidy is defined by a DI greater than 1.0 and hypodiploidy by a DI less than 1.0.

Prognostically significant hyperdiploidy is often measured starting at a DI of greater than 1.16, which corresponds to a modal number of 53 chromosomes. [224] Most cases of ALL exhibit diploidy or hyperdiploidy (Table 19-5). The ploidy of B-lineage ALL karyotypes has long been known to be a prognostic determinant. [221,224,225] Although the absolute number of chromosomes chosen as the "cut-point" for analysis may vary slightly between studies, children with higher ploidy (greater than 50 chromosomes) have the best prognosis. Those in the pseudodiploid category (those with a DI of 1.0 or a normal chromosome number but other chromosomal abnormalities, for example, translocations) have a relatively poor prognosis ( Fig. 19-7). Children with diploidy and hyperdiploidy with 47 to 50 chromosomes have a slightly worse prognosis than that of the hyperdiploid group with 51 to 56 chromosomes, and the best prognosis appears to be for the higher-hyperdiploid group with 56 to 67 chromosomes.[226,227 and 228] Patients in the hyperdiploid group usually share a number of the more important good prognostic features (see the section on prognostic factors), including a favorable age, low initial leukocyte count, and a B-cell precursor phenotype often displaying CALLA. [221] An exception to the general rule that hyperdiploid ALL cases have good prognoses is the relatively rare group of hyperdiploid ALL cases with the near-tetraploid subtype (82 to 84 chromosomes), which appear to have a poorer prognosis.[226,229]

| Ploidy group | Frequency (%) |
|---|---|
| Near haploidy | <1.0 |
| Hypodiploidy, 30–40 | <1.0 |
| Hypodiploidy, 41–45 | 6.0 |
| Pseudodiploidy | 41.5 |
| Hyperdiploidy, 47–50 | 15.5 |
| Hyperdiploidy, >50 | 27.0 |
| Near triploidy | <1.0 |
| Near tetraploidy | 1.0 |
| Normal | 8.0 |

**TABLE 19-5. FREQUENCY OF PLOIDY GROUPS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA CASES**

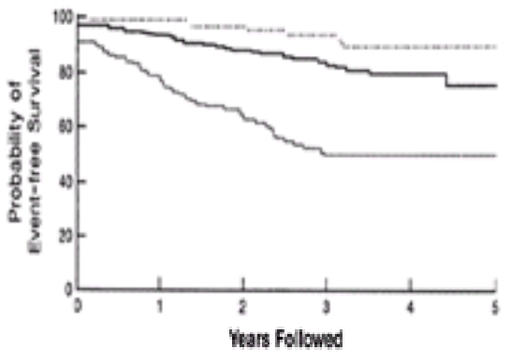


**FIGURE 19-7.** Ploidy is a prognostic determinant. Results are shown for patients with B-precursor acute lymphoblastic leukemia (infants excluded) treated by the Pediatric Oncology Group. Patients (n = 114) with a DNA index greater than 1.16 [i.e., hyperdiploid: (...)] have a better prognosis than those (n = 353) with a DNA index less than or equal to 1.16, white blood cell count (WBC) less than $50 \times 10^9$ per L, and age younger than 11 years; (*bold line*) or a DNA index less than 1.16, WBC less than $50 \times 10^9$ per L, and age older than 11 years (*lowest curve*). (From Trueworthy R, Shuster J, Look T, et al. Ploidy of lymphoblasts is the strongest predictor of treatment outcome in B-progenitor cell acute lymphoblastic leukemia of childhood: a Pediatric Oncology Group study. J Clin Oncol 1992;10:606–613, with permission.)

Hyperdiploidy occurs when there are more than 46 autosomes and two sex chromosomes, so by definition some chromosomes will be present in more than two copies. Trisomy (presence of three copies of a Chromos) is the most common abnormality seen in hyperdiploid ALL. Trisomies of virtually every chromosome have been described in ALL, but the most commonly found include trisomies 4, 6, 10, 14, 17, 18, 21, and X.[228] Trisomies of chromosomes 4 and 10 have been associated with a very low risk of treatment failure in Pediatric Oncology Group studies ( Fig. 19-8).[230] The Children's Cancer Group (CCG) found similar correlations with trisomies of chromosomes 10 and 17 (Fig. 19-9).[228] Trisomy 10 has the larger effect, but in the studies cited, both trisomy 4 and trisomy 17 appear to have independent positive effects on prognosis.[228,230] When the analysis of the combined trisomies 4 and 10 were linked by the Pediatric Oncology Group and combined 10 and 17 by the CCG data, it was found that (in these data sets) the combination of these pairs of trisomies had better outcomes than those with either trisomy alone.[228,230] Trisomy 6 has also been described as a good prognostic karyotypic feature but does not have as strong a positive prognostic correlation as trisomies 4, 10, and 17.[228,231] At least one trisomy, trisomy 5, has been correlated with a slightly worse prognosis in ALL. However, this effect was lost when the T-cell cases were removed from the analysis.[228] It is unclear which genes on any of these trisomic chromosomes may be responsible for the specific biologic behavior and response to individual therapies.

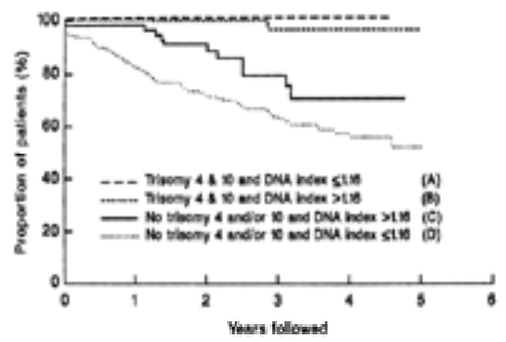


**FIGURE 19-8.** Prognosis of patients with trisomies of chromosomes 4 and 10. Presence of trisomies of chromosomes 4 and 10 are associated with a low risk of treatment failure. Results are shown for patients with B-precursor acute lymphoblastic leukemia (infants excluded) treated by the Pediatric Oncology Group. Patients with these trisomies have a better prognosis than those of patients in the good-risk (DNA index, greater than 1.16) and poor-risk (DNA index, 1.16 or less) groups. (From Harris MB, Shuster JJ, Carroll A, et al. Trisomy of leukemic cell chromosomes 4 and 10 identifies children with B–progenitor cell acute lymphoblastic leukemia with a very low risk of treatment failure: a Pediatric Oncology Group study. Blood 1992;79:3316–3324, with permission.)

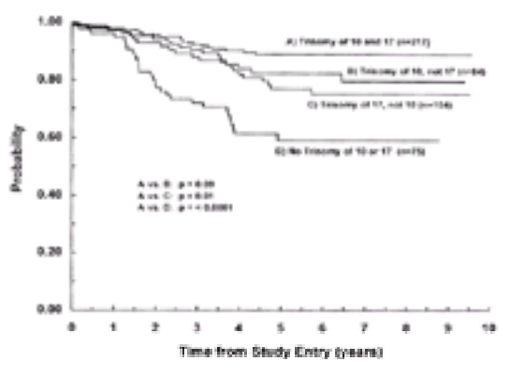


**FIGURE 19-9.** Prognosis of patients with trisomies of chromosomes 10 and 17. Presence of trisomies of chromosomes 10 and 17 are associated with a low risk of treatment failure on Children's Cancer Group protocols. Trisomies of both chromosomes 10 and 17 confer better event-free survival than that for patients with trisomies of each of these chromosomes individually. [From Heerema NA, Sather HN, Sensel MG, et al. Prognostic impact of trisomies of chromosomes 10, 17, and 5 among children with acute lymphoblastic leukemia and high hyperdiploidy (>50 chromosomes). J Clin Oncol 2000;18:1876–1887, with permission.]

Trisomies of chromosomes 8 and 21 are similar to most of the trisomies found in the hyperdiploid category. They are prognostically neutral but warrant some specific comments.[214,232] Trisomy 8, the most common chromosomal numerical abnormality seen in AML, occurs rarely in ALL and is associated with T-cell immunophenotype.[233] When chromosome 8 of leukemic lymphoblasts (i.e., those with trisomies and those without) is examined using FISH, t(8:14)(q24:q32) translocations or duplications of the same 8q24 band may be identified.[232] Region 8q24 is the location of the c-*myc* gene, which is important for cell growth (see Chapter 4 and Chapter 10) and the site of many leukemia-related translocations ( Table 19-1). Thus, the finding of trisomy 8 in leukemic lymphoblasts should prompt a reexamination of the available data to be sure that by morphology, histologic staining, immunophenotype, and karyotype the patient has pre-B ALL and not L3 ALL or AML.

Trisomy 21, as an isolated finding in the leukemic lymphoblasts of non-Down patients, is a neutral prognostic finding.[228] Interestingly, chromosome 21 has been extensively studied and finely mapped, and the sequence was recently published.[234,235] Chromosome 21 contains a large number of known oncogenic transcription and growth factors. AML1, an oncogene from the *runt* family of *Drosophila* transcription factors is encoded on chromosome 21 (21q22). This gene is associated with cytogenetically evident translocations in AML [i.e., the t(8;21) A/ETO fusion] and the t(12;21) TEL/AML1 fusion, which is usually not seen with standard karyotype techniques in ALL (see below) but confers good prognosis.[236,237] and [238]

The worst prognosis (by ploidy) occurs in the rare group of patients with near-haploid ALL (24 to 28 chromosomes), which has an EFS less than 25%.[239,240] Independent of other prognostic factors, ploidy more than or fewer than 45 appears to have important prognostic implications that have not changed with modern therapy (Fig. 19-10).[240,241] Within the fewer than 45 chromosome group, other prognostic factors (i.e., National Cancer Institute standard versus poor risk) still appear

to have some impact (Fig. 19-10).[240]

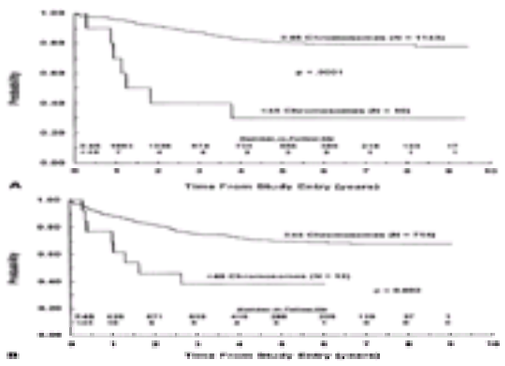

**FIGURE 19-10.** Event-free survival for patients with fewer than 45 chromosomes in their leukemic blasts analyzed by clinical risk grouping. **A:** National Cancer Institute (NCI) standard risk (age 1 to 9 years with leukocyte counts less than 50,000 per µL). **B:** NCI poor risk (age 10 years or older or leukocyte count 50,000 per µL or more). (From Heerema NA, Nachman JB, Sather HN, et al. Hypodiploidy with less than 45 chromosomes confers adverse risk in childhood acute lymphoblastic leukemia: a report from the Children's Cancer Group. Blood 1999;94:4036–4045, with permission.)

### Structural Chromosomal Abnormalities

Structural chromosomal abnormalities also occur in ALL. They are limited to the leukemic cells, a finding consistent with the presumed clonal nature of the disease. Of the structural abnormalities encountered, translocations are the most common. Translocations that are detectable by standard Giemsa banding techniques occur in approximately 40% of cases, but that number is likely to rise with the implementation of the SKY technique described earlier. Multiple recurrent chromosomal translocations have been found in pediatric ALL, which have been linked to rearrangement and altered regulation of cellular oncogenes ( Table 19-1). They are suspected of playing a pivotal role in the leukemogenic process. Translocations are most frequent in the pseudodiploid and hypodiploid groups, occurring with approximately equal frequency in the other abnormal ploidy groups. [213] Translocations were traditionally associated with a poor prognosis. [220,225] The high incidence of translocations in the pseudodiploid and hypodiploid groups may partially explain their relatively poor prognoses. [172] There appears to be an association between certain translocations and immunophenotype (see the section Immunobiology and Table 19-1).

The more common translocations in ALL, the ones linked to prognosis, and known genes in pediatric ALL are listed in Table 19-1. The t(8;14), t(9;22), t(4;11), and t(1;19) translocations are associated with high rates of early treatment failure, but the most common ALL translocation, the t(12;21), appears to have good prognostic implications.

Approximately 5% of ALL patients have visible deletions on standard karyotyping of 12p12-p13 and rearrangements and molecular evidence of loss of heterozygosity involving this region. [238,242,243 and 244] These abnormalities commonly occur in patients with CALLA-positive B-cell precursor disease. [242] The preponderance of reports now indicate that a large percentage of 12p abnormalities in childhood ALL are associated with cryptic t(12;21)(p13;q22) translocations and a good overall prognosis. [244,245] In fact, 22% to 25% of pre-B ALL patients have a cryptic t(12;21)(p13;q22) translocation. This translocation results in the fusion of the coding regions of two transcription factors (i.e., TEL on chromosome 12p13 and AML1 on chromosome 21q22) that are already known to be involved in other hematologic malignancies. [237,244,246]

There is general agreement that the cryptic t(12;21)(p13;q22) confers a favorable initial response to treatment, which extends for at least 3 to 5 years from diagnosis. However, controversy exists concerning the occurrence of late relapses. [247,248,249,250 and 251]

The t(1:19)(q23;p13) translocation, the second most common chromosomal abnormality in childhood ALL, is found in 6.5% of all children with ALL, and is present in 25% of cIg+ pre–B-cell ALL and 1% of cIg early pre–B-cell ALL cases. [213,252,253 and 254] This translocation results in the fusion of the transcriptional activation domain of E2A (a helix-loop-helix transcription factor) on chromosome arm 19p with the DNA-binding homeodomain of PBX1 located on chromosome 1, band q23. [255,256 and 257] The resulting E2A-PBX protein is a transcriptional activator that has been associated with a variety of tumors in different animal models, including T-cell ALL and AML. [258,259] The pre-B ALL cases that have the t(1;19)(q23;p13) and express E2A-PBX1 protein appear to have a poor prognosis. [253,260,261] There appears to be considerable heterogeneity in the location of the E2A and PBX1 breakpoints, however, and in clinical outcome among patients. [253,260,262] For example, patients have been described with a good prognosis who clearly have the t(1;19)(q23;p13) in their leukemic cells but no expression of the E2A-PBX1 fusion protein. It is both the presence of the E2A-PBX1 fusion and its expression in the form of a chimeric messenger RNA and protein that gives the t(1;19) its prognostic influence. [208,263,264] Because the t(1;19) karyotype is often equivocal or falsely negative, molecular techniques such as PCR and FISH have been used effectively to detect E2A-PBX1 fusions. [261,265]

Another fusion partner of the E2A gene on band 19p13 was originally described as a variant of the t(1:19)(q23;p13). [260] The t(17:19)(q22;p13) occurs in 1% of childhood ALL and appears to define a poor-prognosis group of adolescent patients who have an unusual clinical presentation characterized by hypercalcemia, an increased risk of disseminated intravascular coagulation, and a pre-B (cIgM–), low CD10–positivity immunophenotype. [266,267] The E2A fusion partner in this translocation is the hepatic leukemia transcription factor gene (HLF) found on 17q22. The E2A-HLF protein can cause transformation *in vitro*. [268] The known sequence of the E2A-HLF fusion has been incorporated in an RT-PCR system for detecting MRD in patients with this type of disease. [267]

The t(8;14)(q24;q32) can be identified in virtually every case of B-cell ALL (FAB L3). [225,269] In this translocation the c-*myc* proto-oncogene, normally located on chromosome 8, is translocated near a transcriptional enhancer of the immunoglobulin heavy-chain gene on chromosome 14. The resulting dysregulation of c-*myc* expression is believed to be responsible for the uncontrolled proliferation of B cells characteristic of this disorder. In addition to the translocation of c-*myc* coding sequences, mutations sometimes occur in the translocated sequences. [270] Two variant translocations, t(2;8)(p11-p12;q24) and t(8;22)(q24;11), involving the k and l light chains, respectively, are observed less commonly. The similarity in the molecular mechanisms associated with these translocations in B-cell ALL and those that occur in Burkitt's lymphoma supports the presumption that B-cell ALL represents a disseminated form of Burkitt's lymphoma. [219] Patients with B-cell ALL respond quite poorly to conventional ALL treatment but fare somewhat better on therapy similar to that used for Burkitt's lymphoma.

Similar translocations [e.g., t(8;14)(q24;q11)] involving TCR loci and the c-*myc* gene have been described in some cases of T-cell ALL. [271,272 and 273] In these cases, the c-*myc* gene is overexpressed, but the resulting leukemic blasts display T-cell immunophenotype (see the section on T-cell ALL cytogenetics).

There are a number of examples of translocations of transcription factor genes to TCR loci in T-cell ALL ( Table 19-1; see Chapter 4). These translocations may involve the TCRb at 7q34 or the abTCR on 14q11. Rhombotin 1 and 2, TAL1/SCL, TAL2, HOX11, and LYL are the best studied examples of these transcription factor translocations to TCR loci. [274,275,276,277,278 and 279] In most of these translocations, the coding sequences for transcription factor proteins, not normally expressed in T cells, are relocated near the TCR. These translocations cause inappropriate expression of the translocated transcription factors. The mechanisms that lead from the inappropriate expression of these proteins to leukemia presumably vary with the transcription factors involved.

TAL1 (also known as SCL) is translocated to a TCR in approximately 5% of pediatric T-cell ALL cases. A partial TAL1 deletion, which causes overexpression of the TAL1 protein, has been described in approximately 25% of pediatric T-cell ALL cases. [278] TAL1 is not expressed in normal T cells, but it appears to be critical for the formation of the entire hemopoietic system. [280] The mechanism by which TAL1 translocation or partial deletion may cause leukemia is not understood. Translocations involving TAL1, TAL2, LYL, and the TCRs occur in 30% of T-cell ALL ( Table 19-1). [278,281] The genes regulated by these transcription factors are believed to represent a common pathway for the development of T-cell leukemia.

The t(10;14)(q24;q11) and t(7;10)(q35;q24) involve translocations of the transcription factor HOX 11 and TCR loci. HOX genes are known to be important regulators

of hemopoietic development (see Chapter 3 and Chapter 4). Thus, translocations involving different members of this gene family in cases of ALL are not surprising. Despite the heterogeneity of the multiple transcription factor translocations with TCRs in T-cell leukemia, the actual disease associated with these molecular findings is relatively homogenous in clinical and histologic presentation, course, and prognosis. [270,281]

Structural abnormalities, including translocation, deletion, and partial duplication of chromosome band 11q23 are associated with poor prognosis. [282,283] The 11q23 abnormalities are present in 5% to 10% of pediatric and adult ALL, 60% to 70% of infant leukemia (i.e., ALL and AML in patients younger than 1 year), and 85% of secondary leukemias in patients who have received epipodophyllotoxin therapy. [282,284,285,286 and 287] Virtually all of these 11q23 abnormalities have occurred in the same region of a gene variously named MLL (myeloid/lymphoid leukemia gene or mixed lineage leukemia), Htrx1/HRX, and ALL-1. [287,288,289 and 290] The MLL protein is believed to be an important developmental regulator of pluripotent hematopoietic cells. Multiple fusion partner genes have been found for MLL ( Table 19-1 and Fig. 19-11; see also Chapter 3), the most common of which are located on chromosomes 4, 6, 9, and 19.[287,290,291 and 292]

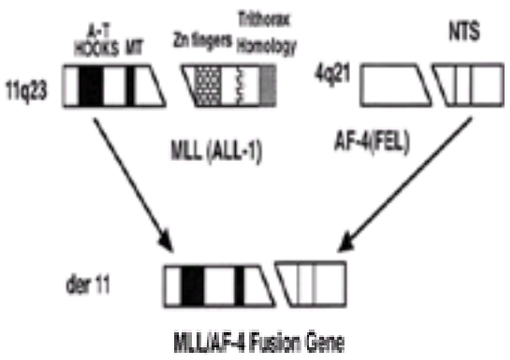


**FIGURE 19-11.** Schematic representation of the MLL/AF-4 fusion protein created by the t(4:11)(q21;q23) found in up to 70% of infant acute lymphoblastic leukemia (ALL) cases. The translocation involves a reciprocal transfer between the ALL-1 gene (located on band 11q23) and the AF-4 gene (located on band 4q21). The resulting der11 product codes for a protein that is thought to be responsible of the development of the 4:11-positive infant ALL. NTS, nuclear translocation signal; MT, methyl transferase activity; Zn, zinc.

ALL patients with rearrangements involving 11q23/MLL have significantly poorer treatment outcomes than do similar patients who do not demonstrate the cytogenetic abnormality (Fig. 19-12).[282,293] The t(4;11)(q21;q23) is the most frequent of these translocations ( Fig. 19-12 and Table 19-1). It has been reported in up to 5% of pediatric ALL cases, is more common in girls, and occurs in more than 60% of infant leukemias of all types. [285,294,295 and 296] The t(4;11)(q21;q23) generally occurs in B-cell precursor ALL and is somewhat more frequently observed in patients with early pre–B-cell disease (cIg+). [285] A large percentage of these cases manifest a characteristic immunophenotype (i.e., CD10–/CD15+/CD19+/CD24+).[285] Leukemic cells from patients with t(4;11) may manifest some cytochemical and ultrastructural features of monocytes and thus have biphenotypic characteristics. Because other translocations involving 11q23 have also been associated with characteristics of mixed lineage [e.g., t(11;19)(q23;p12), t(9;11) (p21;q23)], it has been suggested that leukemias with the 11q23 rearrangement arise from a pluripotent progenitor cell.[213,297]

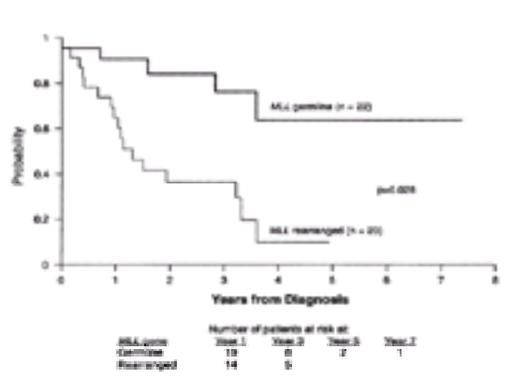


**FIGURE 19-12.** Event-free survival for patients with acute lymphoblastic leukemia, demonstrating the poorer overall prognosis for those with rearrangements involving 11q23/MLL. (From Behm FG, Raimondi SC, Frestedt JL, et al. Rearrangement of the MLL gene confers a poor prognosis in childhood acute lymphoblastic leukemia, regardless of presenting age. Blood 1996;87:2870–2877, with permission.)

The t(9;22)(q34;q11) was one of the first leukemic translocations described and remains the translocation with the worst prognosis in pediatric ALL ( Fig. 19-13).[298,299] The t(9;22) translocation that results in the formation of a small marker chromosome, known as the Ph chromosome, is found in approximately 5% of childhood ALL, and 20% of adult ALL.[270,300] The typical translocation, t(9;22)(q34;q11), is similar to that observed in chronic myeloid leukemia (CML). In the past, it had been suggested that $Ph^+$ ALL represented CML that lacked a chronic phase and presented in blast crisis. Cytogenetic and molecular differences distinguish $Ph^+$ ALL from CML. In $Ph^+$ ALL, unlike in CML, the translocation can usually not be detected in multiple cell lineages. [106,107,301] The Ph chromosome is not detectable during remission in successfully treated $Ph^+$ ALL patients, but it is often present in the remission or chronic phase of CML. [294]




**FIGURE 19-13.** Outcomes for subgroups of pediatric patients with Philadelphia chromosome–positive ALL treated with either chemotherapy alone or bone marrow transplant. **A:** N = 326 patients classified by modified Rome–National Cancer Institute criteria as follows: best prognosis (age 10 years or younger with a leukocyte count less than 50,000 per $mm^3$), intermediate prognosis (intermediate-risk features), and worst prognosis (any age with a leucocyte count greater than 100,000 per $mm^3$). Outcomes analyzed regardless of therapy. **B:** Disease-free survival and overall survival for n = 267 patients treated with either HLA-matched related transplants or chemotherapy alone. (From Arico M, Valsecchi MG, Camitta B, et al. Outcome of treatment in children with Philadelphia chromosome–positive acute lymphoblastic leukemia. N Engl J Med 2000;342:998–1006, with permission.)

The t(9;22)(q34;q11) translocation disrupts the proto-oncogene c- *abl* on chromosome 9 that encodes the tyrosine kinase ABL, which is part of the *ras* signaling

pathway (see Chapter 3). In CML, the c-*abl* gene on chromosome 9 is translocated to a 5.8-kb span of chromosome 22 known as the major breakpoint cluster region (M-bcr).[302] In $Ph^+$ ALL, however, the breakpoints on chromosome 22 usually occur upstream from the M-bcr at a site referred to as the *minor breakpoint cluster region* (m-bcr or bcr-2).[219,303,304] The translocation places the c-*abl* coding sequences under the transcriptional control of breakpoint cluster region (bcr) on chromosome 22. There are also differences between the bcr-abl gene products, expressed in the two diseases. In most cases of $Ph^+$ ALL, a unique p185 bcr-abl protein with tyrosine kinase activity has been observed that is distinct from the typical p210 bcr-abl protein encoded by the chimeric bcr-abl message in CML (see also Chapter 4).[305,306 and 307] However, the p210 bcr-abl protein also has been detected in some cases of $Ph^+$ ALL.[308] Both fusion products have been shown to encode active tyrosine kinases, immortalize cell lines transfected with fusion protein cDNA constructs, and cause leukemias in transgenic mice.[307] In mice, the p185 fusion protein appears to produce more aggressive disease with shorter latencies than those of the p210. This is consistent with differences in the natural history of ALL and CML.

The Ph chromosome has been observed in B-cell precursor ALL and in T-cell ALL. Children with $Ph^+$ ALL tend to be older, have higher initial leukocyte counts, and are more likely to display FAB L2 morphology. Occasionally, leukemic blasts may have the bcr-abl translocation, but they have inadequate cytogenetics, or they do not demonstrate the Ph chromosome. PCR techniques have proven useful in diagnosing bcr-abl–positive, Ph-negative ALL and CML.[309,310] The bcr-abl fusion protein confers the poor prognosis.[311,312] Quantitative PCR and colony assays may also be useful in measuring MRD during the therapy and after bone marrow transplantations (BMTs) for bcr-abl positivity.[309,313]

Clinically, $Ph^+$ ALL patients respond poorly to therapy, having a distinctly lower remission induction rate, a higher frequency of CNS leukemia, and early recurrence of their disease.[314,315] A subgroup of children with $Ph^+$ ALL who also have partial or complete monosomy 7 apparently have an even poorer prognosis.[316] Some cases of $Ph^+$ ALL exhibit mixed-lineage characteristics. It has been suggested that, because of its poor prognosis, children with $Ph^+$ ALL require an alternative to conventional ALL treatment. Early BMT with an HLA-identical sibling, and in some cases with an alternative donor, has been the recommendation of many groups, and the results of BMT appear to have improved over time (see the section on bone marrow transplantation).[315,317,318 and 319] Recently, a subgroup of $Ph^+$ ALL patients has been identified with low-risk presenting features (see the section on prognostic factors) who appear to do well with intensive conventional therapies ( Fig. 19-13).[320,321]

Deletions of chromosomal band 9p21-22 occur in pediatric ALL with a frequency of 10% to 30%.[322,323] The reported incidence of deletions in the 9p21-22 region has increased as the use of Southern blots, PCR, and newer interphase FISH techniques capable of detecting lesions not visible using classic cytogenetic techniques has increased. Two separate regions are the targets of these deletions at 9p22. At one locus, the interferon cluster and interferon-b1 gene are fully or partially deleted. The second locus, at 9p21, is the site of two cyclin D kinase inhibitors, p16 and p15. p16 is a potent tumor suppressor, which has been shown to be important in a variety of adult solid tumors.[323] It is thought that deletion of p16, p15, or both allows cells to progress through the $G_1$ cell cycle check point in an uncontrolled way, ultimately resulting in leukemia (see Chapter 3). Deletions of large portions of 9p (including the 9p21-22 region) are associated with T-cell ALL, but do not (with current therapies) seem to be associated with prognosis.[324]

As previously noted, trisomy 8 and del9p are frequently seen in T-ALL.[324] Other frequently described structural abnormalities in T-ALL include del6q and breakpoints at the known immunoglobin and TCR genes (Table 19-1). The ploidy findings in T-ALL are very different than the ones described above for common pre-B ALL. The majority (86% in one study) of T-ALL are pseudodiploid or normal diploid.[324,325] Despite the description of a series of characteristic cytogenetic features for T-ALL, none of these findings appears to identify patients at greater or lesser risk of relapse. Stratification strategies for T-cell patients generally rely on clinical presentation features, and when they are grouped with pre-B ALL patients with similar findings the clinical results appear to be equivalent.

The reciprocal translocation of the long arms of chromosomes 5 and 14, t(5;14)(q31;q32), has been characterized by a B-lineage phenotype and hypereosinophilia.[326] The immunoglobulin heavy-chain gene and the promotor region of the interleukin-3 (IL-3) gene are joined by this translocation.[213] Overexpression of the IL-3 gene may be involved in the pathogenesis of the hypereosinophilia and leukemia observed in these patients.[327]

Unique translocations, observed in single cases, make up approximately one-half of the chromosomal translocations in ALL.[219] Whether all translocations are intimately involved in the leukemogenic process is unknown. It appears likely that some translocations, through induction of altered gene expression, confer a growth advantage on cells of a particular phenotype.

Chromosomal studies of bone marrow obtained during remission in ALL are karyotypically normal. The presence of aneuploidy during remission usually heralds relapse.[328] At relapse in most patients, the leukemic clone is cytogenetically related to that observed at diagnosis, although evidence of clonal evolution at relapse is common.[213,328] This information is consistent with the suggestion that relapse of ALL signifies the recurrence of original leukemia rather than the development of a new leukemic clone.

### Biochemical Characterization

Various biochemical markers have been studied in ALL. Some have been found to be useful in the diagnosis and classification of the disease; others have been evaluated as potential avenues for selective therapy. Terminal deoxynucleotidyl transferase (TdT) is an unusual DNA-polymerizing enzyme that catalyzes the polymerization of deoxynucleoside monophosphates into a single-strand DNA primer without the need for template instruction.[329] TdT is found in the nucleus and is thought to play a role in immunoglobulin and T-cell antigen receptor rearrangement, influencing the generation of immunologic diversity.[330] Significant TdT activity is not present in normal lymphocytes but is detectable in normal cortical thymocytes and in leukemic lymphoblasts of T-cell and B-cell precursor lineage. TdT activity is usually not present in mature B-cell ALL.[331] Determination of TdT activity may be helpful in the diagnosis of ALL and in differentiating ALL from AML, in which TdT activity rarely occurs.[332] Detection of TdT activity may help identify sanctuary relapses (e.g., testes), particularly in cases in which routine pathologic examination yields equivocal results. Because TdT-positive cells may be present in increased numbers in patients recovering from chemotherapy or BMT, they cannot be used as a sole indicator of bone marrow relapse.[332] Serial measurement of TdT activity in peripheral blood lymphocytes obtained during remission does not permit the earlier detection of bone marrow relapse.[333]

Purine pathway enzymes play an important role in normal lymphocyte function, and this pathway has been extensively studied in ALL.[334] A unique pattern of three enzymes, adenosine deaminase, 5'-nucleotidase, and purine nucleoside phosphorylase, has been observed in ALL.[335,336 and 337] Abnormal lymphocyte function and the absence or reduction in activity of each of these enzymes is a characteristic of certain immunodeficiency disorders. Among the acute leukemias, the activity of adenosine deaminase, which catalyzes the conversion of adenosine to inosine, is highest in ALL. The highest levels are found in T-cell ALL, which is also characterized by decreased 5'-nucleotidase and purine nucleoside phosphorylase activity compared with that of non–T-cell ALL.[329,336,337 and 338] Investigators have attempted to take advantage of the unique biochemical profile of T-cell ALL.[339] A potent inhibitor of adenosine deaminase, 2'-deoxycoformycin, as well as several other purine analogs singly and in combination, have demonstrated activity against a broad spectrum of lymphoid malignancies.[336,340,341 and 342]

Elevated serum levels of lactate dehydrogenase (LDH) have been observed in ALL at diagnosis.[343,344] LDH levels reportedly normalize during remission and increase again at relapse. Abnormalities in lysosomal enzymes also have been observed in ALL.[345] Glucocorticoid receptors have been identified on leukemic lymphoblasts, and the distribution of glucocorticoid receptor number appears to differ significantly among the major immunologic subtypes of ALL. The greatest numbers of receptor sites per cell are seen in early B-lineage ALL. T-cell ALL has significantly lower receptor numbers, and B-cell ALL has the lowest.[346,347] Glucocorticoid receptor content correlates with sensitivity to steroid treatment *in vitro*, and attempts have been made to correlate receptor number with response to therapy *in vivo*. Lower receptor number has been associated with poorer responses to induction therapy and shorter remission durations.[348,349] It is uncertain whether glucocorticoid receptor number is an independent prognostic variable that provides more information than the technically less complex, more conventional prognostic factors.[350] Nevertheless, a poor *in vivo* clinical response to initial corticosteroid therapy has been used by the Berlin-Frankfurt-Munster (BFM) study group to identify patients at particularly high risk for treatment failure.[170,351]

### Pharmacogenetics

In addition to ALL cells having unique biochemical and enzymatic profiles, it is reasonable to assume that individual patients will demonstrate differences in drug metabolism and response, which may have important effects on therapeutic efficacy and toxicity. The field of pharmacogenomics studies the genetic basis for the differences between individual responses to specific drugs.[352,353] Perhaps one of the best examples of pharmacogenomic effects in ALL relates to the isoforms of thiopurine methyltransferase (TPMT). TPMT is a crucial enzyme that metabolizes parent 6-mercaptopurine (6-MP) into an inactive metabolite. Patients homozygous for TPMT null mutations have been shown to have severe 6-MP related toxicity, whereas heterozygote patients appear to have moderate toxicity with 6-MP. Patients

who have two copies of the wild-type TPMT allele show no untoward toxicity when treated with 6-MP.[354,355] Thus, under certain circumstances the toxic effects of a therapeutic drug may be largely dictated by the function of a single gene. However, it is more likely that the pharmokinetic profile of a particular agent in any individual patient is related to a complex interaction between the various alleles of multiple genes and other factors related to therapy (e.g., concomitantly administered drugs, state of hydration). Efforts to control for some of these factors by using rational pharmacokinetic dosing of drugs (see the section Treatment) have shown promise.[356,357]

It appears that some of the biochemical pathways that are implicated in the etiology of the disease (see the discussion of environmental factors in the section Epidemiology) may also be involved in determining the response to and toxicities of various chemotherapeutic agents. The GST genes encode at least four different subfamilies of cytosolic proteins, some of which display known genetic polymorphisms. GST genotypes that confer lower enzyme activities may enhance the efficacy and toxicity of chemotherapy. The enzymatic null genotypes of GST have been associated with a decreased risk of relapse in childhood pre-B ALL and with an increased risk of toxicity and lower overall survival for patients treated for AML.[358] These same GST null mutations have been linked in small series to the development of several adult solid tumors and with pediatric ALL.[25,358]

### Cytokinetics

$^{3}$H-thymidine labeling indices and flow cytometry have been used to evaluate cell kinetics in newly diagnosed patients with ALL.[359,360 and 361] An inverse correlation appears to exist between lymphoblast proliferative capacity and prognosis. Several investigators have shown an association between high proliferative activity and shorter remission duration, suggesting that the kinetic characteristics of leukemic blasts underlie the clinical responsiveness of children with ALL. It has been suggested that the $^{3}$H-labeling index and the percentage of cells in S phase function as independent prognostic variables, but this has not been a consistent finding and awaits further confirmation.[361,362 and 363] Lymphoblast proliferative activity varies with immunophenotype; higher $^{3}$H-thymidine–labeling indices and a greater percentage of cells in the S phase of the cell cycle have been observed in T-cell ALL and B-cell ALL.[360,361] In one study, a poor response to induction treatment correlated with a low RNA content in pretreatment marrow.[363] This finding presumably indicated a greater percentage of nonproliferating cells in these patients.[363,364]

## CLINICAL PRESENTATION

The signs and symptoms of the child presenting with ALL reflect the degree of bone marrow infiltration with leukemic cells and the extent of extramedullary disease spread. The most common symptoms and clinical findings (Table 19-6) are usually manifestations of the underlying anemia, thrombocytopenia, and neutropenia, which reflect the failure of normal hematopoiesis. Pallor, fatigue, bone pain, petechiae, purpura, bleeding, and fever are often present. Lymphadenopathy, hepatomegaly, and splenomegaly are manifestations of extramedullary leukemic spread. Hepatosplenomegaly occurs in approximately two-thirds of the patients and is usually asymptomatic. Lymphadenopathy, usually painless, may be localized or generalized.

| Clinical and laboratory features | Percentage of patients |
|---|---|
| **Symptoms and physical findings** | |
| Fever | 61 |
| Bleeding (e.g., petechiae or purpura) | 48 |
| Bone pain | 23 |
| Lymphadenopathy | 50 |
| Splenomegaly | 63 |
| Hepatosplenomegaly | 68 |
| **Laboratory features** | |
| Leukocyte count (mm$^3$) | |
| <10,000 | 53 |
| 10,000–49,000 | 30 |
| >50,000 | 17 |
| Hemoglobin (g/dL) | |
| <7.0 | 43 |
| 7.0–11.0 | 45 |
| >11.0 | 12 |
| Platelet count (mm$^3$) | |
| <20,000 | 28 |
| 20,000–99,000 | 47 |
| >100,000 | 25 |
| Lymphoblast morphology | |
| L1 | 84 |
| L2 | 15 |
| L3 | 1 |

**TABLE 19-6. CLINICAL AND LABORATORY FEATURES AT DIAGNOSIS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA**

The duration of symptoms in children presenting with ALL may vary from days to months. Anorexia is common, but significant weight loss is infrequent. Bone pain, particularly affecting the long bones, is common and reflects leukemic involvement of the periosteum and bone. Young children may present with a limp or refusal to walk. Bone tenderness is frequently observed. These symptoms and the presence of arthralgias, which may result from leukemic infiltration of a joint, frequently make the delineation between ALL and nonmalignant disorders, such as juvenile rheumatoid arthritis or osteomyelitis, difficult.

T-cell ALL represents approximately 15% of ALL cases and is noted for its distinctive clinical features. It frequently occurs in older boys who present with high initial leukocyte counts and commonly have a mediastinal mass. Approximately one-half of children with T-cell ALL have mediastinal masses, and one-third to one-half have initial leukocytes counts greater than 100,000 per mm$^3$.[365,366] Patients with T-cell leukemia reportedly have a higher incidence of CNS leukemia.[367]

Signs or symptoms of CNS involvement are rarely observed at the time of the initial diagnosis. A number of presenting clinical features and laboratory findings have prognostic importance. These are elaborated in the sections on prognostic factors and treatment.

The child with ALL typically presents with nonspecific symptoms. Thus, ALL may mimic a variety of other nonmalignant (Table 19-7) as well as malignant conditions. These include infectious mononucleosis, idiopathic thrombocytopenic purpura, acute infectious lymphocytosis, pertussis and parapertussis, and certain viral illnesses (e.g., cytomegalovirus and EBV infections), all of which may have similar clinical features. Childhood ALL must be differentiated from other pediatric malignancies that may present with bone marrow involvement, including neuroblastoma, rhabdomyosarcoma, retinoblastoma, and non-Hodgkin's lymphoma. Under light microscopy, neuroblastoma may be difficult to differentiate morphologically from ALL, especially if typical neuroblastoma pseudorosettes are not present. Additional laboratory and clinical evaluation can differentiate these two disorders.

| |
|---|
| Nonmalignant conditions |
| Juvenile rheumatoid arthritis |
| Infectious mononucleosis |
| Idiopathic thrombocytopenic purpura |
| Pertussis; parapertussis |
| Aplastic anemia |
| Acute infectious lymphocytosis |
| Malignancies |
| Neuroblastoma |
| Retinoblastoma |
| Rhabdomyosarcoma |
| Unusual presentations |
| Hypereosinophilic syndrome |

**TABLE 19-7. DIFFERENTIAL DIAGNOSIS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA**

Clinicians must also consider ALL among the differential diagnosis of the rare patients who present with hypereosinophilia. Cases of ALL occurring in association with symptomatic hypereosinophilia have been reported, and in rare instances, eosinophilia has preceded the diagnosis of ALL by many months.[368] In symptomatic patients with eosinophilia, the classic findings of the hypereosinophilic syndrome (i.e., Löffler's syndrome of hypereosinophilia, pulmonary infiltrates, cardiomegaly, and congestive failure) have been observed. The pathogenesis of ALL occurring with hypereosinophilia is not fully defined, but a characteristic translocation, t(5;14), is associated with this syndrome.[326] ALL presenting with hypereosinophilia must be differentiated from eosinophilic leukemia and from AML presenting with

hypereosinophilia, which also has a characteristic chromosomal abnormality involving structural rearrangements of chromosome 16. [369]

**Leukemia or Lymphoma?**

ALL and childhood non-Hodgkin's lymphoma are closely related disorders, and distinguishing between the two can be difficult. Many patients who present with features characteristic of a lymphoma, such as an anterior mediastinal mass, massive lymphadenopathy, or both, have bone marrow involvement. The malignant T cells of lymphoblastic lymphoma are indistinguishable from those of T-cell ALL, and the malignant B cells of Burkitt's lymphoma are similar to those from children with B-cell ALL. B-cell ALL shares immunologic and molecular features of Burkitt's lymphoma and is considered to be a disseminated form of that disease. Most institutions treat patients with advanced B-cell disorders with similar chemotherapy regimens.

The distinction between T-cell ALL and T-cell lymphoblastic lymphoma is also ill-defined. There is some evidence that these disorders arise from different stages of T-cell differentiation and have immunophenotypes reflecting different stages of T-cell maturation. [168] Because this distinction does not apply in every case, it does not provide a reliable basis for delineating between the two diseases.

In the absence of more refined biologic criteria, the percentage of blasts in the bone marrow is conventionally used to differentiate T-cell ALL and T-cell non-Hodgkin's lymphoma. This arbitrary method is confounded by the fact that different institutions and study groups have used different criteria. For some, more than 25% blast cells in a bone marrow signifies leukemia; for others, any evidence of abnormal bone marrow infiltration, regardless of percentage, is used to define leukemia. Because of these differences, meaningful comparisons of treatment results obtained by different groups are often difficult.

**Hematologic Abnormalities at Presentation**

An elevated leukocyte count (greater than 10,000 per $mm^3$) occurs in approximately one-half of patients with ALL. In approximately 20% of patients, the initial leukocyte count is greater than 50,000 per $mm^3$ (Table 19-6). The degree of leukocyte count elevation at diagnosis is the single most important predictor of prognosis in ALL. Neutropenia (less than 500 granulocytes per $mm^3$) is a common phenomenon and is associated with an increased risk of serious infection. [370] Anemia (hemoglobin less than 10 g per dL) exists in approximately 80% of patients at diagnosis. Even if the anemia is severe, the erythrocytes usually manifest a normocytic, normochromic pattern, and the reticulocyte count is low. Thrombocytopenia occurs in most patients; approximately 75% have fewer than 100,000 platelets per $mm^3$. Isolated thrombocytopenia, however, is a rare event. The severity and the degree of bleeding correlate with the degree of thrombocytopenia. [371] Severe hemorrhage is rare even with platelet counts less than 20,000 per $mm^3$ unless fever and infection, both of which can affect platelet survival and function, are present. [371]

Rarely, ALL may initially manifest with pancytopenia and must be differentiated from aplastic anemia. [372,373] An aplastic presentation may represent a true preleukemic state.[372] Other explanations have been reported, including inhibition of normal hematopoietic progenitors by leukemic cells and evidence of an abnormal cellular reaction during or before the aplasia followed by a definitive diagnosis of ALL. [374,375]

To definitively establish the diagnosis of leukemia, a bone marrow aspirate is necessary. Although leukemia cells may be present in the peripheral blood at diagnosis, attempts to establish the diagnosis on the basis of morphologic assessment of these cells alone may be misleading. Under most circumstances, a bone marrow aspirate provides sufficient material to establish the diagnosis. Occasionally, bone marrow biopsy may be required. Although more than 5% lymphoblasts in the bone marrow is highly suggestive of leukemia, a minimum of 25% blast cells is usually required before the diagnosis is confirmed. Usually, most cells in the marrow aspirate are leukemic lymphoblasts. In some situations (e.g., to differentiate an aplastic presentation of ALL from aplastic anemia) multiple bone marrow aspirates and biopsy specimens may be required.

Until recently, definitive diagnosis of the specific leukemic cell type was made primarily by morphologic assessment of bone marrow aspirate slides stained with Romanovsky's dye and by the use of special histochemical stains (e.g., periodic acid–Schiff, myeloperoxidase, Sudan black B), which are helpful in differentiating ALL from AML (Table 19-3).[376] TdT analysis may also contribute important diagnostic information. However, few centers rely solely on morphologic or cytochemical information to make the diagnosis of ALL. Most routinely use immunophenotyping, using a panel of monoclonal antibodies ( Table 19-4) and cytogenetics. Both traditional karyotyping and the use of molecular technologies such as RT-PCR, and FISH (see the section on cytogenetics) are used for identification of chromosomal features. In difficult diagnostic cases, ultrastructural evaluation (i.e., using electron microscopy) and molecular analysis may be helpful.

**Other Abnormal Laboratory Findings**

A variety of other abnormal laboratory study results frequently are obtained in newly diagnosed patients with ALL. Many of these findings and their degree of abnormality reflect the leukemic cell burden, the extent of extramedullary spread, or the excessive proliferation and destruction of the leukemic cells. Increased serum uric acid levels, most common in patients with a large leukemic cell burden, reflect increased anabolism and catabolism of purines. A major complication of hyperuricemia is uric acid nephropathy and subsequent renal failure. The risk of this complication is greatest immediately after the start of treatment, when leukemic cell lysis releases large quantities of uric acid. Adequate hydration, alkalinization, and the use of the xanthine oxidase inhibitor allopurinol have traditionally been required to prevent this potentially serious complication. Alternatively, the high uric acid level can be ameliorated with hydration and the therapeutic use of urate oxidase (an agent commonly used in many countries that is still considered investigational in the United States). [377,378] The only significant side effect of uricozyme noted thus far is a relatively high [4.5% of patients in a St. Jude Children's Research Hospital (SJCRH) study] of acute hypersensitivity reactions. [378] A recombinant preparation of the enzyme is under development, which may alleviate this problem.

Various metabolic abnormalities may be encountered, including decreased and increased serum levels of calcium and increased levels of potassium and phosphorus. Renal stones, both of the $CaPO_4$ and precipitated uric acid types, may occur and are principally treated with hydration. These abnormalities are more frequent in patients with bulky disease (i.e., extensive lymphadenopathy and hepatosplenomegaly), and high initial leukocyte counts. [379] Kidneys may be infiltrated with leukemic cells and are often enlarged at diagnosis. [380,381] Although kidney infiltration and dysfunction can complicate the initial therapy, it does not usually effect outcome. [382] Hypercalcemia may result from leukemic infiltration of bone, although release of a parathormone-like substance from lymphoblasts has been reported. [383] Elevated serum phosphorus levels can occur as a result of leukemic cell lysis and may induce hypocalcemia. [384]

Hepatic dysfunction resulting from leukemic infiltration of the liver is usually mild. Leukemic cell lysis, ineffective hematopoiesis, and liver involvement are associated with elevation of serum LDH. Approximately 5% to 10% of newly diagnosed patients, usually those with T-cell ALL, have an anterior mediastinal mass detected on chest radiographs. Skeletal radiographic changes, particularly in the long bones, are most frequent and include transverse radiolucent metaphyseal growth arrest lines, periosteal elevation with reactive subperiosteal cortical thickening, osteolytic lesions, and diffuse osteoporosis. [385] Radiologically documented bone changes may be seen in asymptomatic patients. When present, bone pain usually resolves quickly after initiation of antileukemic therapy. Rarely, ALL may masquerade as osteomyelitis.[386] Coagulation abnormalities may occur but are usually not a feature of the disease, and at presentation, disseminated intravascular coagulation is encountered infrequently. [387] When coagulopathies occur at diagnosis or early in therapy, they are usually associated with concomitant infection or with therapy, particularly with the use of L-asparaginase, rather than with the leukemia itself. [388] Both *Escherichia coli* and *Erwinia* L-asparaginase may produce thromboses or hemorrhagic infarction.[389,390]

**Patterns of Spread**

Extramedullary involvement may be readily detectable clinically or demonstrable solely by diagnostic tests and procedures. Extramedullary disease is significant because it may cause morbidity at a localized site, and it often represents a significant percentage of the total body leukemia burden. Many patients have some evidence of extramedullary involvement at diagnosis (Table 19-6). The most common sites of extramedullary spread are the CNS, testes, liver, kidneys, nodes, and spleen. However, virtually any site in the body can become involved either at initial presentation or relapse—skin, ocular anterior chamber, pleural and pericardial spaces, and ovarian involvement have all been described. [97,391,392] From a clinical point of view, the two most important sites of extramedullary involvement are the CNS and the testes.

***Central Nervous System Leukemia***

CNS leukemia is found at diagnosis in fewer than 5% of children with ALL. [393] With the institution of CNS preventive therapy, routine lumbar punctures have become an integral part of ALL treatment protocols. As a consequence, symptomatic CNS disease is observed less frequently, and the diagnosis is most often made in the

asymptomatic patient. Diagnosis of CNS leukemia requires cytologic confirmation of leukemic cells in CSF. CSF obtained by lumbar puncture must be examined after cytocentrifugation, a procedure that concentrates the leukemic cells and increases diagnostic sensitivity. [394,395] Leukemic cells found in the CSF are usually cytogenetically identical to those found in the bone marrow. In symptomatic patients, CSF pressure is usually increased, and elevated CSF protein and hypoglycorrhachia are common. With CNS leukemia now more frequently diagnosed in the asymptomatic patient, CSF pressure may be normal, CSF leukemic cell counts relatively low, and abnormalities in CSF chemistry determinations absent.

If there is no significant pleocytosis, the diagnosis of meningeal leukemia is problematic. [396] Controversy exists about the meaning of blast cells in situations in which the total CSF white blood cell (WBC) count is low, such as fewer than 5 WBCs per µL with blast cells. Three separate studies have documented that this circumstance, identified in as many as 19% of patients at initial presentation, was associated with a significantly higher likelihood of CNS relapse when compared with patients without detectable CSF lymphoblasts.[397,398] Other investigators have not been able to confirm this finding. [399,400] In an effort to evaluate the importance of low WBC count, lymphoblast-positive initial CSF samples, National Cancer Institute–sponsored studies are prospectively gathering data on these patients. [401] The consensus criteria for grading CNS involvement at diagnosis are outlined in Table 19-8.[401] CNS-1 status is defined as no evidence of leukemic lymphoblasts in the CSF. CNS-2 and CNS-3 are respectively defined as fewer than 5 WBCs per µL with blasts and greater than or equal to 5 WBCs per µL with blasts (or with cranial nerve palsy) on CSF cytospin preparations. In suspicious but equivocal cases, TdT determination has been suggested as an additional means of confirming the diagnosis of CNS leukemia.[402] The results of cranial computed tomographic (CT) scans, electroencephalography, plain skull radiographs, and magnetic resonance imaging (MRI) may be abnormal for patients with CNS leukemia, but are often normal. None of these imaging methods has sufficient diagnostic sensitivity to be indicated for routine use, but they should be used as symptoms clinically indicate.

| Status | Cerebrospinal fluid findings |
|---|---|
| CNS-1 | No lymphoblasts |
| CNS-2 | <5 WBCs/µL with definable blasts on cytocentrifuge examination |
| CNS-3 | ≥5 WBCs/µL with blast cells (or cranial nerve palsy) |

WBC, white blood cell.

**TABLE 19-8. DEFINITIONS OF CENTRAL NERVOUS SYSTEM (CNS) DISEASE STATUS AT DIAGNOSIS BASED ON CEREBROSPINAL FINDINGS**

CNS leukemia may result from hematogenous spread of circulating leukemia cells or by direct extension from involved cranial bone marrow. [403] Hematogenous spread may occur by means of migration of circulating leukemia cells through venous endothelium or as a consequence of petechial hemorrhages in cases of severe thrombocytopenia.[404] The choroid plexus, with its abundant capillaries, is often a site of leukemic infiltration. [405] Direct extension of leukemia cells may occur from involved cranial bone marrow through bridging veins to the superficial arachnoid. Eventually, infiltration of the deep arachnoid, the pia-glial membrane, and the brain parenchyma itself may occur. Direct spread from involved cranial bone marrow may also occur along the perineurium.

The signs and symptoms of clinically overt CNS leukemia include headache, nausea and vomiting, lethargy, irritability, nuchal rigidity, papilledema, and other manifestations of increased intracranial pressure. Cranial nerve involvement, most frequently involving the third, fourth, sixth, and seventh cranial nerves, may occur with other symptoms or as an isolated event. Infiltration of the optic nerve can result in visual disturbances. Eighth cranial nerve involvement, manifested by hyperacusis, tinnitus, vertigo, and even deafness, has been observed. A more unusual manifestation of CNS leukemia is the hypothalamic obesity syndrome, in which destruction of the ventromedial nucleus of the hypothalamus, the satiety center, results in hyperphagia, pathologic weight gain, and diabetes insipidus. Leukemic subdural involvement and spinal epidural leukemia with spinal cord compression also have been observed. Intracranial leukemic cell masses occur relatively rarely. The numerous neurologic manifestations of overt CNS leukemia make it obligatory for clinicians to exhaustively investigate any neurologic signs or symptoms in the child with ALL to exclude the possibility of CNS leukemia.

The potential clinical impact of CNS leukemia did not become fully apparent until the late 1950s and early 1960s. With improved systemic therapy and longer survival, the CNS became the most common site of initial relapse.[406] By the early 1970s, the incidence of CNS leukemia in some studies ranged as high as 80% to 85%. [406,407] Initial bone marrow remissions of short duration could be obtained in most patients, but CNS recurrence was common. [408] The significance of this increasing rate of CNS relapse was twofold. First, CNS leukemia itself was difficult to eradicate. Second, CNS relapse was usually followed by the rapid development of bone marrow disease.[409] Most patients who experienced a CNS relapse during the era before CNS preventive therapy died as a consequence of marrow relapse rather than of CNS disease. The recognition of this phenomenon led to the development of effective CNS preventive therapy, a strategy in part responsible for the improved prognosis of children with this disease (see the section Central Nervous System Preventive Therapy).

***Testicular Leukemia***

Demonstrable testicular disease is rarely present at initial diagnosis, but occult testicular disease has been diagnosed in 25% of newly diagnosed boys. [410] The possibility of occult testicular disease, together with the fact that testicular recurrence is frequently followed by systemic relapse, prompted several centers to advocate routine bilateral testicular biopsy at some time during maintenance chemotherapy or immediately before its cessation. This practice has been abandoned by most centers because of studies indicating that testicular biopsies at diagnosis, after induction, during maintenance, or before cessation of chemotherapy are associated with a significant false-negative rate and do not accurately predict for eventual testicular relapse. [411,412 and 413] Routine testicular biopsies for occult disease at the end of therapy are no longer recommended. Noninvasive screening for occult testicular disease using transscrotal ultrasound and MRI has been evaluated; neither technique is sufficiently sensitive. [414]

Clinically, overt testicular involvement usually appears as painless testicular enlargement, most often unilateral. The diagnosis must be established by testicular biopsy. When testicular leukemia is suspected clinically, bilateral biopsies are indicated because disease frequently affects the contralateral testis. [415] Wedge biopsies are the preferred diagnostic technique, because this procedure is less likely to result in sampling error. Histologic interpretation is frequently difficult. The incidence of false-negative results from testicular biopsies obtained during maintenance therapy or before stopping all treatment approaches 10%. [412,416] Although it has been suggested that TdT determination may help discriminate between leukemic lymphoblasts and reactive lymphocytes in equivocal biopsy specimens, the value of TdT determination appears to be limited.[417] Immunophenotyping of testicular biopsy specimens may help confirm leukemia cells, but it has not been demonstrated to improve the overall detection rate.[418]

Before the introduction of effective chemotherapy for ALL, clinically evident testicular relapse was a rare event. [419] Paradoxically, with improved therapy and prolonged survival, the incidence of testicular involvement increased. In the 1970s and 1980s, the incidence of overt testicular relapse was reported to be as high as 16%, although the actual figure is probably less than 10%. [420] In boys who had successfully completed a full course of chemotherapy for their disease, overt testicular recurrence was a principal cause of late relapse. The reported incidence in this setting varies but was approximately 10%. [421,422] With the introduction of more intensive therapies, the incidence of testicular relapse may be declining toward 5% or less. [1,4]

The occurrence of isolated testicular relapse led many investigators to consider the testes to be a sanctuary site for extramedullary disease. It has been suggested that leukemic testes are protected from therapeutic concentrations of systemically administered chemotherapy by the blood-testes barrier. [423] Animal studies, however, have questioned the role of the blood-testes barrier in the development of testicular leukemia. [424] The leukemia often can be demonstrated in the liver, spleen, and abdominal lymph nodes in patients studied by exploratory laparotomy at the time of a presumed isolated testicular relapse. [425] This raises the possibility that “isolated” testicular relapse may be a misnomer. Some evidence indicates that the incidence of testicular relapse may be somewhat lower on treatment regimens using more aggressive therapy.[425,426] Although the use of prophylactic testicular irradiation to prevent testicular disease had been advocated, this approach has not gained wide acceptance because testicular relapse has become less of a problem on current protocols, and testicular irradiation is associated with sterility (see the section Late

Effects of Treatment).[427,428]

In testicular relapse, the disease is usually located within the interstitial spaces; in advanced cases, leukemic infiltration of the tubules may occur. [429] Several factors have been associated with an increased likelihood of developing testicular relapse, including a high initial leukocyte count (greater than 20,000 per $mm^3$), T-cell disease, prominent lymphadenopathy and splenomegaly, or significant thrombocytopenia (less than 30,000 per $mm^3$).[429,430] The time to development of overt testicular relapse ranges from 2 months to several years.[420,431,432]

## PROGNOSTIC FACTORS

Certain clinical and laboratory features exhibited at diagnosis, including early response to induction criteria, have prognostic value. The identification of such factors has become essential in the design and analysis of modern therapeutic trials. It is common practice to assign patients on the basis of prognostic factors into different risk groups and to tailor treatment accordingly. Prognostic characteristics of childhood ALL ( Table 19-9) have included the following: the initial leukocyte count, age at diagnosis, sex, race, degree of organomegaly and lymphadenopathy, presence of a mediastinal mass, initial hemoglobin level, initial platelet count, FAB morphologic classification, cytogenetics, immunophenotype, expression of myeloid antigens on leukemic cells, serum immunoglobulin levels, CNS disease at diagnosis, the length of time to attainment of remission, glucocorticoid receptor levels, and HLA type and nutritional status. [136,433,434,435] and [436] The prognostic implications of many of these are covered in the sections on epidemiology, pathogenesis, and pathobiology.

| |
|---|
| Initial white blood cell count |
| Sex |
| Rapidity of leukemic cytoreduction |
| Cytogenetics/ploidy |
| Immunologic subtype |
| French-American-British morphology |
| Mediastinal mass |
| Organomegaly and lymphadenopathy |
| Hemoglobin level |
| Race |
| Platelet count |
| Serum immunoglobulins |
| Myeloid antigen expression on leukemic cells |
| Nutritional status |

**TABLE 19-9. FACTORS ASSOCIATED WITH PROGNOSIS FOR PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA**

Because they are readily available and relatively independent predictors of prognosis, the parameters of initial leukocyte count and age at diagnosis have traditionally provided the most reliable basis for patient stratification. The relative value of other clinical and laboratory features as prognostic indicators varies. To a certain extent, intensity of therapy has become an important prognostic factor. As modern therapy for ALL has become more intensive as well as more successful, many clinical and laboratory features that were once important prognostic features have lost statistical significance with appropriate multivariate analysis. Similarly, differences in treatment approach between large cooperative groups have resulted in differences in published, statistically significant prognostic factors. To facilitate comparisons among the results from different groups, a National Cancer Institute workshop developed a set of consensus prognostic factors outlined in Table 19-10.[401]

| Risk | Definition | 4-year event-free survival (%) | Percentage of B-precursor patients |
|---|---|---|---|
| Standard | WBC count <50,000/μL and age 1.00–9.99 yr | 80.3 | 68 |
| High | WBC count ≥50,000/μL or age ≥10.00 yr | 63.9 | 32 |

WBC, white blood cell.
Note: Table describes the event-free survival and percentages of patients with pediatric B-precursor acute lymphoblastic leukemia treated by the Pediatric Oncology Group (-POG and 1800 series) Protocols by the Uniform Age and WBC Count Criteria.
From Smith M, Arthur D, Camitta B, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. J Clin Oncol 1996;14:18, with permission.

**TABLE 19-10. UNIFORM AGE AND WHITE BLOOD CELL COUNT CRITERIA FOR B-PRECURSOR ACUTE LYMPHOCYTIC LEUKEMIA STANDARD AND HIGH-RISK COHORTS ADOPTED AT THE CANCER TREATMENT AND EVALUATION PROGRAM/NATIONAL CANCER INSTITUTE WORKSHOP**

### Leukocyte Count and Age

The initial WBC and age are the two factors universally accepted as prognostic factors. In most cases initial leukocyte count retains its importance after adjustment for other criteria. There is a linear relation between the initial leukocyte count and outcome in children with ALL; children with the highest leukocyte counts tend to have a poor prognosis.[437,438] Although there is no sharp dividing line and the data are influenced by the specific therapy given, patients with an initial leukocyte count greater than 50,000 cells per $mm^3$ (approximately 20% of children with ALL) are recognized as having a particularly poor prognosis. [439] The biologic basis for higher initial leukocyte counts is unclear, although there are definite associations between certain biologic features and this pattern of presentation. Patients with T-cell ALL often have a high initial leukocyte count and increased lymphoblast proliferative activity.

There is also a relation between age at diagnosis and outcome. [438] Patients who are young at diagnosis (younger than 2 years) and older patients (older than 10 years) have a relatively poor prognosis compared with children in the intermediate age group. The worst prognosis has traditionally been for infants younger than 1 year at diagnosis (see below).[440] Adolescents with ALL also fare poorly, having a lower remission induction rate and EFS than that of other children, excluding infants.[441] Adolescents tend to have a greater constellation of other poor-risk features including T-cell immunophenotype. [441] In the recently published DFCI 91-01 study, age was the only statistically significant prognosis factor. [442] This study included both high- and standard-risk patients (using traditional age, WBC, and karyotype criteria) and demonstrated an EFS for all patients of 83% ± 2% ( $p = .03$). There were a limited number of infants (n = 7 of which six were evaluable) in this study, but they did remarkably well with 5-year EFS of 71% ± 17%.

Infants (i.e., those younger than 12 months of age) with ALL have historically demonstrated poor prognosis (EFS less than 10% to 20%), worse than virtually any other leukemia patient group.[440,443,444] Infants with ALL have an increased incidence of poor prognostic features, including increased initial leukocyte count, massive organomegaly, thrombocytopenia, CNS leukemia at diagnosis, and failure to achieve complete remission status by day 14 of induction therapy. [440] Although their complete remission rate appears to be no different than that of older children, the EFS and disease-free survival (DFS) for patients in this age group has been extremely poor. This is the consequence of a high incidence of early bone marrow and extramedullary relapse. The CNS relapse rate in infants is particularly high.

ALL of infancy appears to be biologically unique. The leukemic cells appear to arise from a very early stage of commitment to B-cell differentiation. They usually express the HLA-DR antigen, are CALLA (CD10) negative, and do not express mature B-cell antigens. [445] Although there are data regarding the immunoglobulin and TCR gene configuration in these patients that suggest ALL in infancy represents an earlier stage of B-cell development than that found in the B-cell precursor ALL of older children, this finding has not been universal. [446] Infants with ALL also have an increased incidence of chromosomal abnormalities that are associated with a poor prognosis (Fig. 19-11 and Fig. 19-12; see the section on cytogenetics). Structural abnormalities of chromosome 11, particularly rearrangement of band 11q23 within the MLL/ALL-1 gene, are observed frequently.[446,447] The t(4;11) abnormality is particularly common. The leukemic cells from infants with ALL frequently express myeloid markers (e.g., CD15), suggesting that in many infants, the leukemia arises in a multipotent precursor cell. [440,446,448] Infants with rearrangement of 11q23/MLL

have a particularly poor prognosis (Fig. 19-12). Because of their poor prognosis, infants are stratified separately for treatment purposes.

Recently, the EFS for infants has been raised to 50% and higher by using high-dose antimetabolites, rotating pairs of non–cross-resistant chemotherapy, and using maintenance similar to standard leukemia therapy.[449,450 and 451] It has been suggested that there may be chemoresponsive subgroups of infants with ALL that can be determined on the basis of initial prednisone responsiveness. [452]

### Cytogenetic Factors

Cytogenetic abnormalities in chromosomal number and structure are common in pediatric ALL (see the section on pathobiology) and appear to have prognostic significance. One interesting association between cytogenetic status and treatment response involves the metabolism of methotrexate. Hyperdiploid leukemic lymphoblasts accumulate increased amounts of methotrexate and methotrexate polyglutamates, and they have higher basal apoptotic rates compared with leukemic cells with lower ploidy and normal cells.[453,454] These may be factors in the better prognosis observed for patients with hyperdiploid lymphoblasts. The prognostic significance of both ploidy and structural cytogenetic changes are presented with the specific associated cytogenetic findings above and in Figure 19-7, Figure 19-8, Figure 19-9, Figure 19-10, Figure 19-12, and Figure 19-13 and Table 19-1 and Table 19-5.

### Sex

The prognostic importance of sex has been well documented (see also the section Epidemiology) and persists on modern chemotherapy protocols.[455,456] In most studies, girls have a better prognosis than boys. Although this appears to be partially due to the development of testicular relapse and to the higher incidence of T-cell disease in boys, there are likely to be other genetic, metabolic, and endocrine effects that contribute to this difference.

### Immunophenotype

T-cell ALL historically has been characterized by significantly shorter remission duration and decreased overall survival. Recent evidence appears to show that the outcome for T-cell patients on intensive chemotherapy regimens has improved and that it may be possible to adequately treat these patients on intensive, short (e.g., 1-year) protocols.[457,458] Because of the frequent occurrence of T-cell disease in older patients with high initial leukocyte counts, the degree to which the T-cell immunophenotype is a significant independent prognostic variable is unclear. [145,434] Younger (especially younger than 5 years) T-cell patients, especially those who present with lower WBC counts and smaller amounts of bulk disease, have done quite well both on the T-cell–specific and more standard therapies of multiple cooperative groups.[459,460,461 and 462]

Whether myeloid antigens on ALL lymphoblasts may have prognostic importance is controversial. Initial reports indicated that concomitant expression of myeloid antigens on the lymphoblasts of children and adults with ALL was associated with a particularly poor prognosis. [188,199] These results conflict with several large studies of children with ALL treated with intensive therapy that found no differences in the rates of complete remission or the EFS between children whose cells expressed two or more myeloid-associated antigens and those with one or no myeloid-associated antigens. [200,201] The different findings in these and other studies of myeloid antigen expression may reflect differences in the definition of myeloid antigen expression, immunophenotyping techniques, or the type of treatment. [202]

### Race

The effect of race on prognosis has been a controversial topic (see the section Epidemiology). It now appears that blacks with ALL present with a different distribution of clinical and biologic features, including a higher frequency of elevated leukocyte counts, mediastinal masses, and L2 morphology. [3] The common ALL phenotype and hyperdiploidy appear to occur less frequently in black ALL patients. This results in proportionately more black children than white children being stratified on higher risk protocols. Hispanic children have a similar increased rate (relative to white children) of higher risk disease, but the effect does not appear to be as large as with black children.[3] Within the various risk categories, it appears that Hispanic children have equivalent outcomes as those of white children, but black children may be doing slightly worse. This appears to be related to variations in chemotherapeutic response, which may be due to specific differences in pharmacogenetic profiles (see the section Pharmacogenetics).[3]

### Leukemic Cell Burden

Leukemic cell burden can be assessed indirectly by evaluating the extent of extramedullary disease. The degree of hepatosplenomegaly and lymphadenopathy have emerged in most univariate analyses as important prognostic variables. Although in some studies multivariate analyses reveal that some of these features, such as a mediastinal mass, may tend to be dependent variables, other features retain significance even after adjustment for other independent variables, such as the initial leukocyte count. The BFM study group has used a measurement of hepatosplenomegaly with the initial leukocyte count to compute a “risk factor index,” which forms (along with response to glucocorticoids) part of the prognostic basis for subsequent treatment stratification. [463]

### Response to Treatment

Rapidity of response to treatment has emerged as an important (perhaps the most important) indicator of prognosis. It has been known for a long time that patients who do not achieve a complete remission within the usual 4- to 6-week induction period have a high rate of relapse and shortened survival. [464] A number of studies have shown that assessment of initial response at earlier time points yields valid prognostic information. Residual leukemia demonstrable in bone marrow on day 14 of induction is an independent predictor of outcome.[464,465] Patients with residual disease on day 14 have a lower rate of complete remission and a greater likelihood of early (within the first 24 months of therapy) and late relapse.[465] The day 7 marrow status also has been correlated with treatment outcome.[466] One group reported that the failure to clear peripheral leukemic blasts by day 8 of therapy correlates significantly with a poorer 5-year EFS. [467] Patients who have an intermediate response to initial chemotherapy (i.e., M1 by day 14) do less well but have a superior EFS compared with slow responders (i.e., M1 status by day 28). [468] Rapid early responders (i.e., those with an M1 marrow on day 7) have the best EFS (Fig. 19-14). A recent report suggests that early response to induction in infants may not have the same prognostic implications that it does for older patients (Vora et al. ASH 2000 [Abs] 1996).

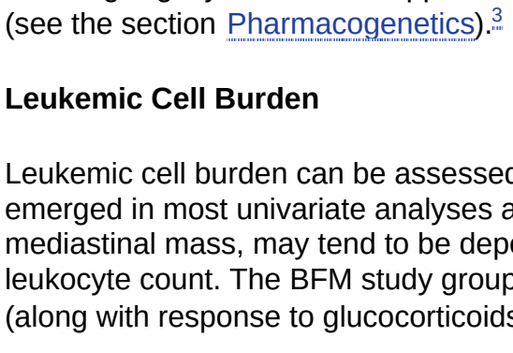


**FIGURE 19-14.** Event-free survival (EFS) and rapidity of cytoreduction during induction treatment. Rapid early responders (RER), those whose bone marrow aspirate was rated M1 by day 7, have the best EFS. Patients who are M2 or M3 on day 7 can be separated by their day 14 marrow results into an intermediate response group (IER) (M1 on day 14) and a slow early response (SER) group. (From Steinherz PG, Gaynon PS, Breneman JC, et al. Cytoreduction and prognosis in acute lymphoblastic leukemia—the importance of early marrow response: report from the Children's Cancer Group. J Clin Oncol 1996;14:389–398, with permission.)

### Nutritional Status

Some studies have suggested that nutritional status is a significant prognostic factor. [469,470 and 471] In one study, undernutrition, defined as both height and weight for

age below two standard deviations, was a significant predictor of treatment failure. [469] In another study, in which weight for age was used to define undernutrition, more than a threefold higher 5-year DFS was observed in well-nourished children. [472] It has also been reported that children who were undernourished have less tolerance for chemotherapy and received suboptimal doses.[473] Additional, confirmatory studies to define the prognostic role of nutritional status are needed.

### Other Prognostic Factors

Low serum levels of IgG have been associated with induction failure. Low levels of all three of the major immunoglobulin subclasses (i.e., IgG, IgM, IgA) have been correlated with a poor chance of EFS; the association with low IgM levels appears to be most significant. [474,475] Most of the immunoglobin findings were detected on earlier series of studies. An association between certain HLA types and prognosis has been suggested but has not been demonstrated in most major studies. The association of specific HLA influences in the development of ALL and the increased incidence of ALL in boys appear to be valid (see the section Epidemiology).[22,23,435,476] The relation between FAB morphologic classification and prognosis was addressed earlier (see the sections on pathobiology and morphologic classification).

### Analyzing Prognostic Factors for Patient Stratification

As therapy has improved, the vast majority of factors, which had prognostic significance on earlier studies, has been rendered insignificant. Until recently, only age, initial WBC count at diagnosis, and a few cytogenetic abnormalities (noted above) appeared to retain their importance. With the newest results of intensive multi-agent chemotherapy, the factors that may retain the greatest prognostic significance appear to be related in large part to therapy itself—that is, early response, MRD at various time points, and the presence of focused cytogenetic (i.e., trisomy 4 and 10) and molecular cytogenetic [t(12;21)] findings in the blasts. [442]

## TREATMENT

The recognition that ALL is a heterogeneous disease and that children can be stratified into various risk groups has profoundly influenced therapy. Although combination chemotherapy remains the primary therapeutic modality, it is no longer considered appropriate, in the context of current biologic knowledge, for all patients with ALL to be treated on a single treatment regimen. The initial evaluation of the patient with ALL requires sophisticated laboratory techniques to derive appropriate cytogenetic, immunologic, or molecular information. As our understanding of the disease has improved, the approach to its treatment has become more complex. This circumstance, coupled with the increased intensity of many current treatment regimens, emphasizes the need for children with ALL to be evaluated and treated by established pediatric cancer centers at which state-of-the-art treatment protocols are available. Although the specific approaches to patients in various risk groups may be somewhat different, modern ALL treatment regimens divide therapy into four main treatment elements: remission induction, CNS preventive therapy, consolidation, and maintenance therapy.

### Induction Therapy

The aim of initial ALL treatment is induction of remission. By definition, patients in remission have no evidence of leukemia when evaluated by physical examination as well as by standard laboratory examination of the blood (e.g., complete blood cell count) and bone marrow with standard cytochemical stains under light microscopy. Peripheral blood values must be within the standard range of normal for age, and the bone marrow must be of normal cellularity, with fewer than 5% lymphoblasts.[477] Complete remission status also assumes the absence of detectable CNS or extramedullary disease by traditional light microscopy on CSF and physical examination findings. [478] Achievement of a traditionally defined remission is a basic premise of antileukemic treatment and a known prerequisite for prolonged survival.[479] In clinically overt ALL, the leukemic cell burden is estimated to be approximately $10^{12}$ leukemic cells (Fig. 19-15).[479,480] To induce a complete remission, chemotherapy must reduce the total number of leukemic cells by 99%, leaving fewer than $10^{10}$ blasts.[481] In reality, most patients who achieve a successful remission induction have their total body leukemia burden reduced even further. [111] As noted above, the rapidity of this response to initial chemotherapy as well as the total reduction in leukemic cell burden are also important factors in determining eventual treatment success (see the sections Prognostic Factors and Minimal Residual Disease).[482,483] Early results with standard risk patients from the CCG, suggest that intervention with intensification of chemotherapy (both in the induction and later consolidation phases) may be able to rescue slow responders so that their EFS is comparable to those with more rapid responses (Stork L.C. ASH 2000 [Abs] 2007).

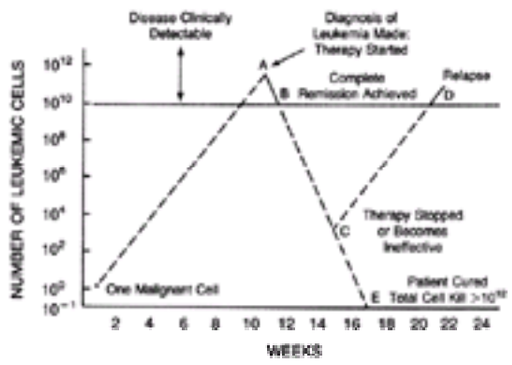


**FIGURE 19-15.** Schematic representation of the results of therapy in a patient with leukemia. (Adapted from Valeriote F, Vietti TJ, Fernback DJ, eds. Clinical pediatric oncology. St. Louis: CV Mosby, 1977:1182.)

Although the basic two-drug combination of vincristine and a glucocorticoid induces remissions in approximately 85% of children with ALL, the addition of L-asparaginase, an anthracycline, or both, improved the remission induction rate to approximately 95%. [484] Prednisone and prednisolone have been the most common glucocorticoids used for this purpose. Recently several cooperative groups have switched to dexamethasone because of laboratory data implying that it is more potent and may have pharmacokinetic advantages (e.g., increased efficiency in crossing the blood–brain barrier) in treating CNS disease. [485,486] However, newer experience suggests that dexamethasone may be associated with higher acute and long-term complication rates, so its use in this setting should remain as part of authorized clinical trials.[487,488] The addition of L-asparaginase to vincristine and a glucocorticoid also significantly prolonged remission duration. [489] Whether the added leukemic cytoreduction theoretically achieved by including a fourth induction (an anthracycline) leads to improvement in remission duration is controversial. The results of one randomized study indicated that adding daunorubicin (daunomycin) to the three-drug combination of vincristine, prednisone and L-asparaginase did not improve remission duration.[490] However, protocols using this four-drug induction combination with intensive consolidation and maintenance therapy uniformly demonstrate improved overall remission duration, even for high-risk patients. [491,492] Because the use of a fourth drug or additional drugs may increase the incidence of toxic effects during induction therapy, many centers reserve the use of drug combinations using four or more agents for patients in the highest risk groups.

Failure of induction therapy is a relatively rare event, occurring in less than 5% of children with ALL treated with most current regimens. [5,493] Induction failure occurs when the patient demonstrates residual leukemia in the end-of-phase bone marrow aspirate (typically performed on day 28 or 36, depending on the regimen), or when there is clear evidence of tumor progression (e.g., rising numbers of leukemic blasts in blood, bone marrow, and extramedullary sites) on chemotherapy. The long-term EFS for induction-failure patients in a recent Dana-Farber Cancer Institute (DFCI) study was 16%. [493] Rarely, a patient has severe aplasia at the end of induction and hence does not meet the criteria for achieving M1. In the DFCI study, prolonged aplasia had a better prognosis than did induction failure and was associated with EFS comparable to that of the patients who were clearly in a standard remission at the end of induction. [493] Patients who have bone marrow aplasia at the end of induction should receive adequate blood product and other supportive measures until the marrow either recovers (M1) or enough blasts can be distinguished (M3) that refractory disease is diagnosed.

There are occasional patients who demonstrate an M2 marrow (greater than 5% but less than 25% leukemic blasts) at the end of a standard induction. Although many of these patients eventually go into remission (either with an extension of the standard induction or with institution of more intensive treatments), this slow response to initial therapy is indicative of poor prognosis (see the section Minimal Residual Disease).[111,494] Improved supportive care has decreased the mortality rate during induction therapy to approximately 3% or less.[5,495]

## Central Nervous System Preventive Therapy

The recognition that CNS recurrence constituted a major obstacle to overall treatment success stimulated efforts to prevent CNS disease. The concept of CNS preventive therapy is based on the premise that the CNS acts as a sanctuary site in which leukemic cells, undetected at diagnosis, reside protected by the blood–brain barrier from therapeutic concentrations of systemically administered antileukemic drugs. According to this view, prevention of CNS relapse is more appropriately called *presymptomatic CNS therapy* than *CNS prophylaxis*, a term that is widely used. The introduction of CNS irradiation as preventive therapy was based on murine studies that demonstrated cures of L1210 leukemia when cranial radiation was added to systemic treatment with cyclophosphamide. [496] The first documentation of the value of CNS preventive therapy evolved from a series of studies performed at SJCRH. Although relatively low doses of craniospinal radiation (500 or 1,200 cGy) demonstrated no preventive effect (studies I–III), the administration of 2,400 cGy cranial irradiation plus five concurrent doses of intrathecal methotrexate or 2,400 cGy of craniospinal irradiation alone (studies V–VII) reduced the incidence of CNS relapse from more than 50% to approximately 10%. [497] Because the spinal component of craniospinal irradiation is associated with excessive myelosuppression and retardation of spinal growth, cranial irradiation (2,400 cGy) plus intrathecal methotrexate became the standard form of CNS preventive therapy.

Although this approach was universally adopted in the 1970s, concerns subsequently developed about the apparent adverse effects of this form of CNS preventive therapy (see the section on late effects of treatment). The identification of brain abnormalities on CT scans, altered intellectual and psychomotor function, and neuroendocrine dysfunction in patients treated with 2,400 cGy of cranial irradiation and intrathecal chemotherapy prompted a reappraisal of CNS preventive therapy strategies and stimulated the search for alternative methods of CNS preventive therapy. [498,499,500,501] and [502] The use of a lower dose of cranial irradiation (1,800 cGy) with intrathecal methotrexate appears to be as effective as 2,400 cGy and is currently used in most CNS preventive therapy regimens that continue to administer cranial irradiation. [394,503]

It is unclear, however, whether the use of 1,800 cGy plus intrathecal methotrexate reduces the incidence of adverse CNS sequelae. There is evidence that 1,800 cGy may have significant negative effects on neurocognitive function. [504,505] One study reported that children with ALL treated with 1,800 cGy of cranial irradiation and intrathecal methotrexate demonstrated greater adverse neuropsychological effects than those treated with intrathecal methotrexate alone. [506] In one German study, the use of 1,200 cGy administered on a protocol that included intermediate-dose methotrexate and systemic reinduction therapy was found to provide as effective CNS preventive therapy as 1,800 cGy in a selected group of standard-risk patients. [507] Although this experience suggests that under some circumstances it may be possible to reduce the cranial irradiation dose even further, additional follow-up, including information about the incidence of adverse CNS sequelae using such an approach, is needed.

A variety of patient characteristics are associated with an increased risk of CNS leukemia, including a high initial leukocyte count, T-cell disease, very young age, thrombocytopenia, lymphadenopathy, hepatomegaly, splenomegaly, and black race. [394] The highest rates of CNS relapse occur in infants and patients with extremely high leukocyte counts or lymphomatous presentations. [394,440] Although no single factor or group of factors can predict with absolute certainty whether an individual patient is at risk for CNS relapse, the recognition that patients differ in their risk for developing CNS leukemia has permitted investigators to successfully modify CNS preventive therapy accordingly. Cranial irradiation is unnecessary for patients with a good prognosis; intrathecal methotrexate alone, given periodically throughout maintenance chemotherapy, provides adequate CNS preventive therapy for these patients. [394] Maintenance intrathecal triple chemotherapy, the combination of intrathecal and moderate-dose intravenous (i.v.) methotrexate, and high-dose i.v. methotrexate alone all appear to provide equivalent protection to that offered by cranial irradiation and intrathecal methotrexate for patients at an intermediate risk of CNS relapse. [508,509]

With current CNS preventive therapy regimens, the incidence of CNS relapse is less than 10% overall and below 5% for good-risk patients. [510,511] and [512] The intensity of systemic chemotherapy appears to influence the efficacy of CNS preventive therapy regimens.

The Associazione Italiana di Ematologia ed Oncologia Pediatrica and the CCG groups have both demonstrated that, provided intensive systemic therapy is used, intrathecal methotrexate alone, administered from the start of treatment throughout maintenance therapy, is equivalent to 1,800 cGy of cranial irradiation in intermediate-risk and even high-risk patients (who were rapid early responders during induction). [513,514] Maintenance intrathecal methotrexate was significantly less effective than 1,800 cGy of cranial irradiation when less intensive systemic chemotherapy was used. [515]

CNS preventive therapy may be associated with acute or subacute neurotoxic sequelae. Intrathecal methotrexate or cranial irradiation alone can produce a wide spectrum of neurotoxicities. [501,516] Intrathecal methotrexate may be associated with an acute arachnoiditis, characterized by headaches, nausea and vomiting, meningism, and other signs of increased intracranial pressure occurring 12 to 24 hours after intrathecal injection. [517,518] These reactions usually are not severe, are self-limited, and have been reduced in frequency by the use of an intrathecal methotrexate dosing schedule based on the relation of age to CNS volume. [519] A subacute form of methotrexate neurotoxicity characterized by varying degrees of encephalopathy, myelopathy, and even paraplegia has been observed more rarely. [520,521] Studies using frequent pulses of intermediate-dose methotrexate together with triple intrathecal therapy reportedly are associated with an increased incidence of neurotoxicity, including seizures and CT scan abnormalities. [522] Between 5 and 7 weeks after cranial irradiation, some patients develop a subacute neurotoxic reaction characterized by somnolence, lethargy, anorexia, fever, and irritability. This “somnolence syndrome,” which may be accompanied by electroencephalographic abnormalities and CSF pleocytosis, usually reverses within 1 to 3 weeks. [523,524] It is unclear whether the somnolence syndrome correlates with the development of other late sequelae seen with CNS preventive therapy using cranial irradiation.

The desirability of avoiding cranial irradiation in CNS preventive therapy regimens results from concerns that radiotherapy is in large part responsible for many of the long-term adverse CNS sequelae observed in patients treated with cranial irradiation and intrathecal chemotherapy. Although patients at low and intermediate risks of CNS relapse may receive equally effective therapy with the alternatives to irradiation previously discussed, many centers continue to administer cranial irradiation plus intrathecal methotrexate to patients at particularly high risk for CNS relapse. Nevertheless, at the present time only approximately 10% to 15% of ALL patients (those in high-risk categories) receive cranial irradiation. This is a marked improvement over the 100% use of this modality in the late 1960s and early 1970s. Additional clinical trials evaluating efficacy and relative toxicity are needed to explore whether cranial radiation can be safely removed from CNS preventive therapy in regimens for high-risk patients.

## Consolidation and Maintenance Therapy

After complete remission has been achieved, subsequent therapy is required. Early studies demonstrated that without additional therapy, most patients relapse within a median of 1 to 2 months. The actual duration of an unmaintained remission varies with the intensity and duration of the induction therapy. [525] As shown in Figure 19-15, patients in complete remission theoretically have a leukemic cell burden in the range of $10^{10}$. Although successful induction may have produced a 99% (two log) or greater reduction in the number of leukemia cells, a significant amount of additional therapy is necessary before the leukemia is totally eradicated.

Using a variety of methods, including cytomorphology, biochemistry, *in vitro* cell culture, cytogenetic analysis, flow cytometry, and molecular biologic approaches such as Southern blot and PCR analysis of immunoglobulin and TCR gene rearrangements, investigators have documented that occult leukemic disease is often present in patients during otherwise apparent remission (see also the section Minimal Residual Disease). [111,526,527,528,529] and [530] At relapse, lymphoblasts usually demonstrate chromosomal and immunoglobulin gene rearrangement patterns identical to those obtained at the time of original diagnosis. [531,532] Several mechanisms have been proposed to explain this persistence of leukemic cells during remission. These include the development of biochemical drug resistance, the residence of leukemic cells in physiologic or pharmacologic sanctuary sites (e.g., CNS, testes), and the maintenance of a population of leukemia cells in a kinetic state ($G_0$) in which they are less vulnerable to chemotherapy.

To effectively prevent relapse, postinduction therapy must suppress leukemic growth and provide continuing leukemic cytoreduction without permitting the emergence of a drug-resistant clone. In early clinical studies, a variety of single agents were evaluated as maintenance agents. [497,533] Drugs particularly effective as induction agents were not useful for maintenance therapy. In contrast, maintenance treatment with methotrexate and 6-MP substantially prolonged remission. [534,535,536] and [537] The combination of methotrexate and 6-MP, administered continuously, has been used most widely and constitutes the principal element in most maintenance therapy regimens. The optimal schedule of administration of these two drugs is different. Methotrexate is more effective administered intermittently, but daily administration of 6-MP appears optimal. Various combinations have been studied in which other agents are added to standard 6-MP and methotrexate regimens. Addition of intermittent pulses of vincristine and prednisone to 6-MP and methotrexate maintenance chemotherapy appears to have prolonged remission duration for some patients, although the value of this approach after intensive induction therapy is unclear. [351,538,539] The choice of appropriate maintenance chemotherapy appears to

differ according to risk group. 6-MP and methotrexate may provide adequate maintenance therapy for certain good-risk patients, but more intensive maintenance therapy appears to be more effective for poor-risk patients.

Consolidation therapy is a period of intensified treatment administered immediately after remission induction, and it is a common component of many therapy protocols, particularly for higher-risk patients. [538,540,541] Several regimens use agents and schedules designed to minimize the development of drug cross-resistance. The evidence that intensification has improved treatment success, even in patients with a poor prognosis, is substantial. [4,542] Intensive postinduction treatment with L-asparaginase and doxorubicin is associated with an improved outcome for high-risk patients with T-cell disease. [543]

The BFM study group, using intensive induction and consolidation plus "reinduction" and "reconsolidation" phases of therapy early in maintenance, reported prolonged EFS for approximately 70% of patients, including those with high-risk features. [4,491] Using a similar approach, the CCG reported an EFS rate of approximately 60% for high-risk patients.[492] The use of high-dose methotrexate pulses also has been reported to be associated with an improved EFS of approximately 73% for children with T-cell leukemia. [468] A protocol known as the *New York regimen*, which incorporated many of the features of the $LSA_2L_2$ lymphoma regimen and used alternating non–cross-resistant combination chemotherapy, has also resulted in an improved outcome for children with high-risk ALL. [542,544,545] An SJCRH study using early reinforcement of induction therapy followed by a rotational combination chemotherapy approach produced an EFS rate of 69% in high-risk patients.[542] Although the use of more intensive treatment regimens has been associated with a modest increase in therapy-associated toxicity, the advantages of such treatment particularly for the patients in the highest risk groups, are established.

Drug dosage is an important factor in maintenance chemotherapy. The correlation between chemotherapy dose and therapeutic response in leukemia was originally documented in animals.[546] Its influence during maintenance was demonstrated in a randomized study in which patients receiving chemotherapy with 6-MP, methotrexate, and cyclophosphamide at full dose had significantly longer remission duration than patients receiving therapy at one-half dose. [478] Subsequent clinical studies have confirmed this finding.[547] One study demonstrated a correlation between cumulative 6-MP dose and prognosis in children with average-risk ALL. [548] A Danish study reported a lower relapse rate among patients who had lower leukocyte counts while receiving maintenance therapy with 6-MP and methotrexate, suggesting that more intensive therapy with these two agents is beneficial. [549] In another study, patients who tolerated only low doses of 6-MP because of neutropenia and those who received higher doses of methotrexate had a lower rate of relapse, indicating that the use of these agents in maximally tolerated doses during maintenance may be associated with improved treatment outcome.[550] The results from several cooperative group trials testing this question (i.e., standard versus dose intensive 6-MP/6-thioguanine/methotrexate) are not yet available

The frequency of drug administration also appears to influence the length of remission. [547] Patients who receive maintenance therapy on a continuous rather than an interrupted schedule have longer remission durations. Studies of the clinical pharmacology of orally administered 6-MP and methotrexate have documented that their bioavailability after oral administration may be limited and highly variable, suggesting a possible explanation for treatment failures that occur during oral maintenance therapy with these agents.[551] Compliance problems also may diminish the efficacy of maintenance therapy. [552] The possible problems associated with oral administration of 6-MP and methotrexate theoretically could be circumvented by parenteral administration. However, data on the relative effectiveness of parenteral or oral maintenance therapy are conflicting. Results of a British Medical Research Council (MRC) leukemia trial (UKALL-VII study) indicated a significantly longer actuarial relapse-free survival rate for patients who received methotrexate intramuscularly compared with those given the drug orally. [444] In another study, which randomized children with non–T-cell ALL to receive methotrexate during maintenance as a single oral dose or as an intramuscular injection, the route of administration appeared to have no real influence on relapse rate. [553]

Some studies indicate that intracellular metabolism of 6-MP and methotrexate may influence treatment outcome. [547,554,555] Higher intracellular levels of 6-thioguanine nucleotides, the major cytotoxic metabolites of 6-MP, have been associated with a lower relapse rate. [554,555] As noted earlier, studies have demonstrated that the lymphoblasts from children with hyperdiploid, low-risk ALL more efficiently accumulate methotrexate intracellular metabolites, indicating that these patients may be more sensitive to methotrexate therapy.[556,557] An association has also been found between high levels of lymphoblast dihydrofolate reductase and shorter remission duration.[558]

***Duration of Treatment***

The optimal length of maintenance chemotherapy has not been established. Most centers treat patients for a total of approximately 2.5 to 3.0 years. Data from several studies support this approach.[559,560,561] and [562] The MRC examined the effect of variation in length of treatment on duration of remission and demonstrated that 19 months of therapy was less effective in preventing relapse than 3 years of treatment. [563]

The optimal duration of treatment for girls may be different than that for boys. Another MRC study demonstrated that 1.5 years of therapy was sufficient or girls but inadequate for boys.[564] The tendency for a greater percentage of males to relapse after cessation of chemotherapy was observed in a long-term follow-up study of patients treated on the Children's Cancer Study Group 141 protocol. In that study, patients in complete continuous remission for 3 years were randomized to discontinue therapy, to receive a 4-week course of reinduction chemotherapy and then discontinue therapy, or to continue therapy for a total of 5 years. No significant difference was found in DFS for the different treatment regimens. However, a higher incidence of late relapse occurred among boys, even after excluding patients with occult testicular disease. This is consistent with other studies that have demonstrated that sex is a significant predictor of late relapse, even when isolated testicular relapse is excluded.[565,566]

Investigators at SJCRH have demonstrated that after successful completion of 2.5 years of therapy, approximately 80% of patients remain free of disease. [566] Most of the 20% of patients who eventually relapsed did so in the first year off therapy. In the second through the fourth year after cessation of chemotherapy, the risk of relapse was only about 2% to 3% per year. Recurrence after 4 years from the cessation of therapy was not encountered. [566] Similar results were observed in a study from Great Britain that concluded that patients alive 6 years after diagnosis without relapse have a high likelihood of prolonged survival and cure. [553]

It is likely that the intensity of therapy has a bearing on the optimal duration of therapy. The current practice of treating patients for 2.5 to 3.0 years of maintenance chemotherapy is derived from studies in which patients were treated with a variety of chemotherapeutic regimens, many of which incorporated fewer agents and were less intensive than those currently in use. For this reason, conclusions about the duration of maintenance based on those studies may not be directly applicable to current treatment programs. It is logical to question whether patients receiving more intensive therapy earlier in their course of treatment may ultimately require a shorter overall duration of therapy. In an attempt to address this question, the BFM study group, which uses an intensive chemotherapy regimen, randomized patients to receive 18 or 24 months of total duration of therapy. A therapeutic advantage was observed for patients who received longer treatment. [351] The MRC UKALL-VIII trial, using less intensive therapy, observed no outcome advantage between patients randomized to receive 3 years and those receiving 2 years of maintenance treatment.[547]

**Supportive Care**

Optimal management of the child with ALL requires appropriate attention to several areas of supportive care, including the rational use of blood component therapy, an aggressive approach to detection and treatment of infectious complications, careful attention to the metabolic and nutritional needs of the patient, and comprehensive, continuous psychosocial support for patient and family. Because these topics are thoroughly addressed in Chapter 39, Chapter 40, Chapter 41, Chapter 42, Chapter 43, Chapter 44, Chapter 45, Chapter 46, Chapter 47, and Chapter 48, they are discussed only briefly here.

The importance of adequate hematologic supportive care cannot be overemphasized. Before the systematic use of platelet transfusions, hemorrhage was the leading cause of death for patients with this disease. The use of prophylactic platelet transfusions and aggressive platelet transfusion support has markedly reduced the incidence of significant bleeding. Recently, the use of 20,000 per $mm^3$ threshold for platelet transfusion in adult leukemia patients (primarily with AML) has been questioned by several researchers. These groups found that the use of 10,000 per $mm^3$ in the absence of fever, trauma, or reason for clotting factor consumption resulted in 20% to 30% less transfusion exposure and cost and no significant increase in serious hemorrhage. [567,568] and [569] However, these studies were done on relatively small numbers of patients that included very few children. The development of recombinant thrombopoietin has raised hope that this stimulator of platelet production may soon play a role in ameliorating chemotherapy-induced thrombocytopenia. Clinical trials of this agent and other cytokines (IL-3 and IL-11) to stimulate postchemotherapy platelet recovery have been initiated. [570,571] and [572]

Erythrocyte transfusions are frequently required to treat anemia, and as with all blood products, they must be appropriately screened to exclude the possibility of

contamination with hepatitis virus or HIV. Modern blood banking and transfusion techniques, including the irradiation of all cellular blood products, WBC depletion, and more comprehensive viral and donor screening has improved the safety and efficacy of transfusion of these children. The granulocytopenia that occurs as a consequence of therapy-induced marrow hypoplasia or with disease progression places patients at risk for potentially life-threatening infections.

Hematopoietic growth factors, including granulocyte colony-stimulating factor (G-CSF), are beginning to play a significant role in reducing the complications of granulocytopenia after cancer chemotherapy. G-CSF has already been shown to ameliorate the leukopenia incurred by chemotherapy for ALL. [573] The long-term benefits of using growth factors during intensive phases of ALL therapy remains controversial. [574,575] Receptors for G-CSF and other known factors (e.g., granulocyte-macrophage colony-stimulating factor, IL-3) have been identified in leukemic lymphoblasts, raising concern that their use might stimulate leukemic cell growth.[576] For these reasons, as well as the significant financial cost, G-CSF and other growth factors are not routinely used in most standard risk ALL regimens, but they are used often in BMT and relapse and occasionally in front-line high-risk protocols that use more intensive chemotherapy. [577]

An aggressive approach to diagnosis and rapid empiric therapeutic intervention are important principles for the successful management of the severely neutropenic (less than 500 granulocytes per $mm^3$) patient (see Chapter 41). The early empiric use of broad-spectrum antibiotics has dramatically reduced overall mortality. Granulocytopenia, chemotherapy-induced immunosuppression, disruption of normal anatomic barriers by invasive procedures, or therapy-induced complications (e.g., mucositis) increase susceptibility to bacterial, fungal, viral, and parasitic infections. Although the risk of bacterial and secondary fungal infections may be greater for patients undergoing intensive induction or reinduction therapy, the child with leukemia is susceptible to various forms of infections while undergoing maintenance chemotherapy as well. *Pneumocystis carinii* pneumonia is an extremely serious, potentially life-threatening complication that commonly affects children undergoing maintenance chemotherapy. The prophylactic use of trimethoprim-sulfamethoxazole, instituted early in therapy, dramatically reduces the incidence of this type of infection and is used routinely in most centers. [578,579] When trimethoprim-sulfamethoxazole cannot be tolerated due to count suppression or allergy, dapsone or pentamidine (i.v. or aerosolized) can be substituted. [580,581]

The leukemic child undergoing treatment is also at risk for disseminated varicella if exposure to an infected person occurs. Administration of zoster immune globulin within 72 to 96 hours to such patients appears to have a protective effect. [582] Immunization of children with ALL with a live, attenuated varicella vaccine has been advocated by some but may not work in many patients because of the immune suppression caused by the disease or chemotherapy. [583,584] The immunization of varicella-susceptible household contacts of patients with ALL who have not had varicella is currently a recommended practice. [585] Other viral infections also place the leukemic child at risk. Measles tends to run a more complicated, atypical course in the leukemic host. [586] Nonimmunized children exposed to the measles virus should be treated with gamma globulin. Because of the risk of dissemination, immunization against measles or the use of any vaccines containing a live virus, except possibly in the case of varicella vaccine, is contraindicated in patients receiving chemotherapy.

Adequate nutrition also is a concern for the patient with leukemia. Multiple studies have suggested that malnutrition is an adverse prognostic factor (see the section on nutritional status and Chapter 42). [469,470,473,587] Severe malnutrition was shown in one study to increase the risk of death 2.5-fold during induction. [471] It has been reported that improving nutrition in undernourished patients during chemotherapy improves prognosis. [587] It may be that this effect is due to the relationship between nutritional status and the ability to tolerate standard maintenance chemotherapy. [588] Undernutrition is common at diagnosis, and it frequently becomes worse during the intensive phases of chemotherapy.[589] In most centers, if normal enteral alimentation is prevented by an unforeseen therapeutic complication, i.v. hyperalimentation is considered.

## TREATMENT OF RELAPSE

Before treating any type of relapse, it is helpful to ascertain whether the cells are indeed from the original leukemia (see also Late Effects of Treatment). Most relapsed leukemias retain their original immunophenotypes and karyotypes, but changes have been observed. [169,173,590] The presence of a different cell lineage at the time of relapse, a so-called lineage switch (e.g., lymphoid to myeloid), is a relatively rare event. Lineage switch may result from chemotherapeutic eradication of one clone and subsequent expansion of a second clone of an originally biclonal leukemia. Alternatively, chemotherapy may induce modulation of antigen expression in a leukemic clone that retains the potential for lymphoid and myeloid differentiation. Lineage switch must be differentiated from the development of a secondary leukemia. Molecular and cytogenetic studies may be helpful in documenting similarity with the original clone. In approximately 30% of relapse cases, the karyotype will have undergone changes visible with standard Giemsa banding. [169] However, there is usually evidence of the original clone within the predominant clone at relapse.[169] Lineage switch usually occurs within months of the initial diagnosis; secondary acute leukemias tend to occur years later. [199] Lineage shift from lymphoid to nonlymphoid disease is more common than the reverse, although both have been described. [191,591] In general, both secondary leukemias and relapse of the original leukemia represent disease that is more resistant to chemotherapy, and relapse protocols (often including at least the consideration of BMT) are more intensive than those used on most de novo cases.

### Bone Marrow Relapse

Bone marrow relapse is the principal form of treatment failure in patients with ALL. The two approaches to the treatment of bone marrow relapse are chemotherapy and BMT. Depending on initial chemotherapy regimen used, second complete remissions can be induced in most patients who experience a marrow relapse. The chemotherapeutic approach to the relapsed patient should include aggressive multidrug reinduction therapy followed by intensive systemic consolidation and maintenance chemotherapy. The combination of vincristine, prednisone, and L-asparaginase produces complete remissions in approximately 70% to 75% of patients treated for an initial bone marrow relapse. The addition of daunorubicin increases the reinduction rate to 80% to 90%. With more intensive regimens, reinduction rates greater than 90% have been reported. [592,593] Administration of an intensive course of cytarabine and teniposide, or high-dose ifosfamide with etoposide induces complete remissions in approximately one-third of patients not achieving complete responses with the four-drug reinduction regimen of L-asparaginase, vincristine, daunorubicin, and prednisone. [594] Wherever possible, new agents (drugs not used in the initial therapy) and new combinations of agents should be introduced to try to overcome any drug resistance the leukemic clone may have acquired during the first treatment course.

At the time of a marrow relapse the CNS must also be checked (with a lumbar puncture) and consideration given to how, if the CNS is not involved, a second course of CNS preventative therapy should be administered. Alternatively, if the CNS is involved at the time of relapse, a plan for active CNS treatment must be developed. The need for a second course of CNS preventive therapy for patients in second remission is well established; without it, almost 50% experience CNS relapse. [595] For patients who received cranial irradiation as part of their initial CNS preventive therapy, intrathecal chemotherapy is usually used as the second form of CNS preventive therapy.[595]

The eventual outcome of children who achieve a second remission is influenced by a variety of factors. Patients relapsing after completion of a previous chemotherapy regimen have a better chance of achieving and maintaining a prolonged second remission than do those relapsing while receiving chemotherapy. [596,597 and 598] The overall survival of patients who relapse off therapy is better than for those who relapse on therapy. The prognosis of patients who relapse off treatment is proportional to the interval from the time of discontinuation of therapy to relapse. In most studies, bone marrow relapse occurring during treatment or within 6 months after cessation of treatment is associated with a very poor long-term survival. [593,599,600] Children whose first relapse occurs later than 6 months after cessation of therapy have significantly longer second remissions. [600,601] One study has suggested that with respect to survival, the primary dividing point was 3 months after discontinuation of therapy.[597] In that analysis, patients who relapsed between 3 and 6 months after cessation of treatment had a relatively good prognosis similar to those who relapsed between 6 and 12 months after therapy, although the best overall survival was observed in patients who relapsed more than 12 months after discontinuation of therapy.

The length of the first remission is a factor with predictive value for remission duration of the second remission. [598,602,603 and 604] Children whose first bone marrow remission was longer than 18 months have a significantly better prognosis than those with shorter first remission durations. A review of 600 children in second remission treated with chemotherapy documented prolonged second remissions in fewer than 5% of children who relapsed within 18 months of achieving first remission. In contrast, sustained second remissions were observed in approximately 25% of children whose relapse occurred after 18 months. [603] Using an intensive reinduction treatment regimen, the BFM study group has observed prolonged second complete remissions in approximately 50% of patients who had prolonged first remissions and who had Ph-negative blasts.[605] For relapsed patients with $Ph^+$ disease, the long-term survival was only 8% in the same study. [605]

Features other than duration of the first remission have been associated with the likelihood of achieving a prolonged second remission, including a low leukocyte count (less than 20,000 per $mm^3$) both at initial diagnosis and relapse, and a favorable age (older than 2 years or younger than 10 years) at initial diagnosis. [602] The nature and intensity of previous therapy appears to be important; patients previously treated with suboptimal primary induction and maintenance therapy have a

higher reinduction rate.[606,607] For patients experiencing multiple relapses, the reinduction rate declines with each successive relapse, presumably a result of the development of drug resistance.[608]

A bone marrow relapse portends a poor prognosis for most patients. With aggressive treatment, however, long-term second remissions are being observed.[596,602,609,610] The results of a number of studies suggest that prolonged second remissions (greater than 2 years) can be obtained with aggressive chemotherapy in approximately 10% to 30% of patients who relapse on therapy and in up to 50% of patients who relapse after elective cessation of therapy.[596,598,603,611,612]

The use of BMT as a therapeutic approach for ALL patients who have bone marrow relapse or are assessed to be at an extremely high risk of relapse (e.g., Ph $^{+}$ at diagnosis) has increased in recent years. This approach, discussed in Chapter 16, involves the administration of intensive cytoreductive therapy, usually using total body irradiation (TBI) combined with high-dose chemotherapy in doses lethal to normal bone marrow, and subsequent hematopoietic "rescue" with i.v.-infused bone marrow obtained from a compatible donor. To some degree, the initial interest in BMT was generated by the unfavorable treatment results obtained in relapsed patients treated with conventional chemotherapy. The earliest experiences with BMT involved multiply relapsed patients refractory to conventional chemotherapy who were usually transplanted in relapse. Results in this group of patients were disappointing; approximately 10% of those receiving marrow from an HLA-matched sibling achieved prolonged DFS.[613,614]

Although these earlier results were discouraging, continual innovation and technological refinements have given BMT an important role in the treatment of childhood ALL. Currently, allogeneic BMT is routinely advocated for patients in second or subsequent remission who have an appropriate HLA-matched sibling donor.[615] In this group of patients, overall DFS ranges from approximately 40% to more than 60%.[616,617] Although it is a matter of some debate (see Chapter 16), some groups are reporting results equivalent to these with matched unrelated donors.[618,619] Other strong indications for BMT (even in first complete remission) include patients with unfavorable cytogenetics [e.g., t(9;22), t(4;11)] or ploidy (haploidy or tetraploidy) of the leukemic blasts, who also have prognostically poor presentation features or who did not respond rapidly to their initial treatment.[620] The value of BMT for infants [with or without the t(4;11) translocation] is controversial and is the subject of ongoing clinical trials. Although non-TBI containing regimens are often chosen for young children, they do not seem to be as effective as the TBI-containing regimens.[617] The optimal preparative regimen for any of the common relapse ALL circumstances (i.e., TBI versus non-TBI containing regimens as well as the choice of chemotherapy agents and doses), alternative donor type, and the issues surrounding the management of graft-versus-host disease (GVHD) and posttransplant care are discussed in detail in Chapter 16.

After a bone marrow relapse, better results are obtained for patients transplanted in second remission than in those transplanted in relapse or partial remission. In most studies patients transplanted in earlier remissions fare significantly better than patients transplanted after multiple relapses.[519,621] The length of first remission and high-risk features at diagnosis are predictive factors.[603,622] Patients with shorter initial remissions and those with high-risk features at diagnosis fare worse when transplanted in second remission. Most studies that have compared the efficacy of allogeneic BMT with chemotherapy for patients who had experienced a previous relapse indicate that transplantation is associated with a superior outcome.[623,624]

A retrospective analysis performed by the International Bone Marrow Transplant Registry compared BMT versus chemotherapy for children with ALL in second remission and concluded that transplantation was superior to chemotherapy in patients whose initial relapse occurred within 18 months of achieving first remission.[603] Allogeneic BMT is considered the treatment or choice for patients in second bone marrow remission who relapsed initially while undergoing chemotherapy (or within 3 months of its completion) and have a histocompatible sibling.[597,603,621] It has been suggested that patients who suffer late relapses (greater than 36 months after achieving first remission or after completing maintenance therapy) or had swift initial response to their initial chemotherapy may do well with intensive chemotherapy regimens without transplant.[320,519] Many centers treat patients whose relapses occur 1 year or more off chemotherapy with chemotherapy alone.[602,624] In such patients, BMT is reserved for subsequent relapse.

Routine allogeneic BMT is limited to the approximately one-fourth of relapsed patients who have an HLA-identical sibling. In an effort to circumvent this problem, several alternative strategies have been studied. One approach is autologous BMT in which pretransplant preparative therapy is followed by infusion of previously harvested remission marrow treated *in vitro* with drugs, immunotoxins, or specific monoclonal antibodies to remove potentially contaminating leukemic cells.[622,625,626] and [627] The results of studies of autologous purged and unpurged BMT used in second or subsequent remission vary, with DFS figures ranging from approximately 13% to 40%.[622,627,628] Whether autologous marrow can be adequately purged is still a matter of debate, but evidence shows that autologous marrow is often contaminated with residual leukemia cells, and that the risk of relapse post-BMT is directly proportional to the number of malignant cells reintroduced in the infused bone marrow.[629] Methods to artificially mark the infused graft have been described and are useful for determining whether the source of a later relapse is from cells that were infused with the graft or cells that survived the BMT preparative regimen.[630,631]

Other alternative donor options for ALL patients who lack a histocompatible sibling include transplantation from a partially matched related donor, a matched or partially mismatched unrelated donor, or an HLA-similar cord blood stem cell donor.[628,632] Techniques such as depleting donor marrow of T cells and the use of better immunosuppressive posttransplant regimens (e.g., low-dose methotrexate, cyclosporine, or FK506; see Chapter 16) to decrease the incidence and severity of GVHD have made these types of transplants feasible. Unfortunately, many of these techniques may also lead to graft rejection and potential decreases in the graft-versus-leukemia effect.[633] The results of these approaches have been encouraging, but they remain in an early stage of clinical development.[628] Alternative donors, autologous purging strategies, better methods to prevent GVHD and other BMT complications, and their possible role in ALL treatment are discussed in more detail in Chapter 16.

The outlook for patients who relapse after BMT is poor. Although complete remission can be obtained in as many as 50% of patients, the duration is usually short.[634]

**Minimal Residual Disease**

Although there may be some changes in immunophenotype and karyotype, in most cases of ALL, relapse represents the reappearance of the original clone.[169,635] Thus researchers have known for a long time that some amount of MRD had to exist below the level of detection. Using light microscopy and routine bone marrow stains it is difficult for even an experienced observer to distinguish less than 5% blasts in a specific bone marrow sample. This is made even more difficult at the end of any intensive treatment (e.g., induction, intensification, consolidation) because the cells in the marrow will be repopulating, and normal hematopoietic precursors (so-called hematogones) will be present in amounts greater than or equal to 5% and can be easily confused with leukemic blasts.[111]

Numerous techniques to detect MRD and predict clinical outcome have been evaluated. The techniques used include various types of quantitative PCR or RT-PCR, cell culture/soft agar cloning techniques, FACS analysis for abnormal immunophenotypes, or combinations of these.[111,635,636] and [637] The earliest approaches had limited success because they lacked both sensitivity and specificity and were relatively cumbersome and expensive to apply.[528,634,638,639] PCR technology greatly enhances the ability to detect residual leukemic cells and is the most sensitive method. Targets for PCR detection include leukemia-specific translocations and clonal antigen receptor or immunoglobulin gene rearrangements that are specific to a particular leukemic clone. The leukemia cell–specific changes can be targeted at the DNA level with PCR or at the level of gene expression with RT-PCR. Using *in vitro* dilution experiments, the PCR approach can detect as few as one cell in $10^5$ to $10^6$ normal bone marrow cells.[636,640,641] When this approach has been practically applied to the clinical evaluation of bone marrow specimens, however, sensitivities of $10^{-4}$ to $10^{-5}$ are more commonly reported.[528,642] The $10^{-4}$ and $10^{-5}$ sensitivity is also within the range of current FACS equipment, which can detect the complex immunophenotype patterns that allow leukemic cells to be clearly distinguished from normal cells. FACS has the added advantage of detecting intact cells, in contrast to PCR and RT-PCR, in which contaminating nucleic acid material from dead cells can complicate interpretation.

Early studies suggested that PCR may be useful in identifying patients likely to relapse when the number of detectable leukemic cells in their marrow seemed to be rising.[528,529] In Ph$^{+}$ ALL, the detection of PCR-positive bcr-abl transcripts after BMT or intensive conventional therapy were the first cases in which increasing amounts of PCR+ cells were found to be associated with a decreased DFS.[643,644] More recently the best use of this technology appears to correlate with what is already known about the prognostic value of rapid response to chemotherapy (see the sections Treatment and Prognostic Factors). It is becoming clearer that the rapid (i.e., within the first 2 to 4 weeks) lowering of MRD levels to below $10^{-4}$ correlates well with relapse-free survival.[111,645,646] Conversely, patients in whom MRD does not clear below this point, or more slowly goes below the same level have a higher risk of relapse.[111]

Multiple issues remain to be clarified in prospective studies, including whether MRD levels below $10^{-5}$ are relevant and the most appropriate times during therapy for

MRD measurements. Once these correlative studies are done, it will hopefully be possible to develop interventions to prevent relapse in patients in whom the MRD analysis predicts are at high risk of recurrence. At this point, clinicians should be aware that MRD analysis and its eventual applicability to the clinic are still under investigation. A PCR-negative result during or at the end of therapy does not necessarily guarantee long-term DFS. Conversely, although it has been demonstrated that many patients who are cured appear to be PCR negative, it is not clear that achievement of a PCR-negative status is necessary for cure in all cases.

## Extramedullary Relapse

### *Central Nervous System Relapse*

Despite the success of CNS preventive therapy in dramatically reducing the incidence of CNS recurrence, CNS relapse remains a significant cause of treatment failure in ALL. CNS recurrence is observed in less than 10% of patients. In the past, the outcome for these patients was generally poor, with most patients suffering subsequent CNS relapses or recurrences at other sites, such as bone marrow and testes. [394,647] CNS relapse may occur as an isolated event, with a bone marrow relapse, or with recurrence at another extramedullary site (e.g., testes). Because periodic lumbar puncture surveillance for CNS leukemia is performed in most ALL treatment protocols, it is relatively infrequent for patients to present with overt CNS symptoms. More commonly, the diagnosis of meningeal recurrence is based on a routine examination of CSF. The criteria for diagnosing a CSF relapse have been modified over the years. [395] The generally accepted criteria has included more than 5 leukocytes per µL, with unequivocal blasts demonstrable in a cytocentrifuge preparation. Although this has been a useful working definition, the significance of blast cells in a cytocentrifuge sample when the CSF leukocyte count is less than or equal to 5 leukocytes per µL is unclear. A study by Odom and colleagues [648] suggested that positive, low cell count CSF samples obtained on surveillance lumbar punctures performed during remission indicate a high likelihood of impending CNS relapse. A CCG study, however, did not confirm these findings.[649]

Intensive treatment plans have recently improved the results for patients with an isolated CNS relapse as high as 70% EFS. [650,651] There is evidence that if both craniospinal irradiation and intrathecal chemotherapy are used as CNS prophylaxis in the context of moderately intensive systemic therapy during the initial treatment regimen, the rate of CNS relapse can be reduced to as low as 1% to 2%. [652] However, the combination of CNS and radiation therapy prophylaxis has worse intellectual and growth long-term effects, so except in specific high risk situations, combined-modality treatment for CNS prophylaxis is reserved for relapse situations. [516,653,654]

The most successful treatment regimens for CNS relapse have used intrathecal chemotherapy for CNS remission induction, followed by consolidation therapy with craniospinal irradiation, and maintenance intrathecal chemotherapy. [651,655] Intrathecal methotrexate alone induces CNS remissions in more than 90% of patients; however, unless followed by maintenance intrathecal therapy or craniospinal irradiation, relapse occurs within 3 to 4 months. [656] Therapy for CNS relapse must also contain vigorous systemic therapy to prevent further relapses of all types. Cranial irradiation alone has little value in the treatment of overt CNS disease because it does not adequately treat sites of disease along the spinal axis. Furthermore, when cranial irradiation is combined with intrathecal methotrexate alone, it appears to increase toxicity and does not appear to prolong remission duration. [409,656] Craniospinal irradiation alone, administered in adequate doses (2,400 cGy to both sites) can induce complete remissions and prolong DFS. Most centers, however, do not use craniospinal irradiation alone. The higher spinal irradiation dose required to achieve equivalent disease control if intrathecal therapy is omitted is associated with prolonged bone marrow suppression, which compromises the ability to deliver adequate systemic chemotherapy. A common approach is first to induce a CSF remission with intrathecal chemotherapy and then to administer craniospinal irradiation at doses of 2,400 to 3,000 cGy to the cranial vault and 1,200 to 1,800 cGy to the spinal axis. [394,657] The main factors determining the success rate for patients with CNS relapse include whether the relapse occurred greater than or equal to or less than 18 months (83% and 46% EFS, respectively; Fig. 19-16) after initiating therapy and whether the patient received CNS-directed radiation therapy in their initial treatment regimen. [650,651]

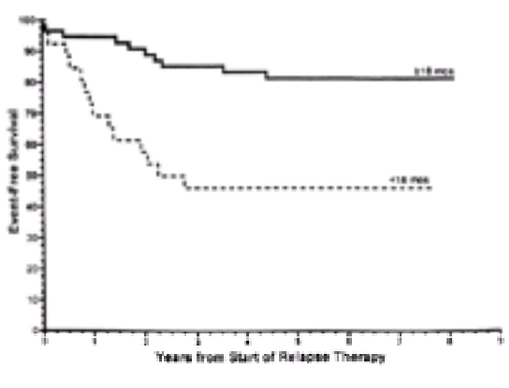


**FIGURE 19-16.** Event-free survival (EFS) for patients who have isolated central nervous system (CNS) relapses greater than or equal to or less than 18 months from initial diagnosis. The 4-year cumulative EFS rate for patients with first remission at greater than or equal to 18 months was 83.3% ± 5.3%, and, for patients with a first remission at less than 18 months, it was 46.2% ± 10.2%. For all patients (n = 83) the EFS after a first isolated CNS relapse was 71.1% ± 5.3%. (From Ritchey AK, Pollock BH, Lauer SJ, et al. Improved survival of children with isolated CNS relapse of acute lymphoblastic leukemia: a pediatric oncology group study. J Clin Oncol 1999;17:3745–3752, with permission.)

The role of craniospinal irradiation to treat CNS recurrence in a patient who originally received cranial irradiation as part of CNS preventive therapy is less certain. Results from the MRC Concord and UKALL-I trials demonstrated a continuous complete remission rate of less than 10% for these patients. [658] Other investigators have demonstrated substantially better results with this approach. [657] However, craniospinal irradiation administered in this setting is known to pose a significantly greater risk of delayed neurotoxicity.[657,659]

Several attempts have been made to improve on the use of single-agent intrathecal methotrexate for CNS relapse. Triple therapy consisting of simultaneously administered intrathecal cytarabine, hydrocortisone, and methotrexate has been advocated, but this produces remission durations similar to those achieved with methotrexate alone.[394] The use of intraventricular chemotherapy, administered by means of an intraventricular subcutaneous reservoir, more completely distributes drug within the CNS, minimizes patient discomfort, and avoids the problems of inadequate delivery of drug into the CSF that may occur with unsuccessful lumbar punctures.[660] Early studies demonstrated longer remission durations and fewer CNS relapses with intraventricular than with intralumbar therapy. [659,661]

Several other treatment approaches have been advocated for controlling meningeal leukemia. High-dose systemic methotrexate infusions induce CSF remissions in most patients.[662] The feasibility or effectiveness of this approach in maintaining CNS remissions is unknown, and its applicability may be limited, particularly in patients receiving CNS irradiation, because of concerns about delayed neurotoxicity. [394,663] Systemic high-dose cytarabine also has produced remissions in patients with overt CNS leukemia.[664,665] The use of high-dose cytarabine is somewhat limited by its attendant myelosuppression and neurotoxic potential. New intrathecal agents are also being developed. Diaziquone, mafosfamide, and topotecan are all being evaluated in separate, ongoing intrathecal phase I or II clinical trials. [660,666] The eventual role of these and other agents in the prevention and treatment of CNS leukemia requires further study.

### *Testicular Relapse*

With the increase in intensity of many protocols, the incidence of testicular relapse appears to be decreasing from the 10% to 15% seen in the 1970s and 1980s to 2% to 5% on more recent trials.[1,4,667,668] Optimal therapy for testicular relapse includes the administration of local radiotherapy and the use of systemic chemotherapy. Radiation dose appears to be a crucial factor in local control. Doses less than 1,200 cGy are generally suboptimal; doses of 2,400 cGy to both testes have been considered adequate.[432,669] Reports of local recurrence in patients treated with 2,400 cGy, however, suggest that higher doses may be better. [670] Bilateral testicular radiotherapy is indicated for all patients; unilateral treatment may be followed by relapse in the contralateral testis. [420]

Radiation therapy adversely affects normal testicular function. Sterility is an expected consequence at the radiation doses used. [432,671,672] Studies also indicate that testicular endocrine function may be impaired at doses of 2,400 cGy. Elevated follicle-stimulating hormone and luteinizing hormone levels, decreased testosterone levels, and delayed sexual maturation have been observed after gonadal irradiation. For this reason, such patients must be carefully followed for signs of delayed

sexual maturation and may require androgen replacement therapy.[673,674]

The impact of a testicular relapse on prognosis depends on whether it was overt (clinically detectable) or occult (detected on routine testicular biopsy), whether the recurrence was an isolated event or accompanied by a simultaneous hematologic relapse, and whether the relapse occurred during or after initial treatment. [420,597,675] Because isolated testicular relapse frequently heralds a systemic relapse, treatment must include intensification of systemic therapy in addition to bilateral testicular irradiation.[429,676] Most centers systemically "reinduce" patients who suffer an overt testicular relapse with intensive systemic chemotherapy. This strategy has dramatically improved the prognosis for patients with testicular relapse. [415,676,677] and [678]

The prognosis is better if the testicular relapse occurs as an isolated event. [420] Isolated testicular relapse is observed more frequently than it is with a concurrent bone marrow relapse, although many patients who present with isolated testicular relapses probably have occult intra-abdominal disease. [425] The outcome for patients with an isolated overt testicular recurrence appears to vary with the time of presentation. An isolated testicular relapse occurring in a patient on treatment is associated with the worst prognosis, although a CCG study suggests that with local irradiation and intensive systemic retreatment, prolonged EFS can be obtained in nearly one-half of such patients.[676] In contrast, a late, isolated, overt testicular relapse that occurs off therapy has an even better prognosis. Prolonged DFS can be obtained for more than two-thirds of such patients.[411,415,675]

The practice of elective testicular biopsy during apparent remission can detect microscopic testicular leukemia in approximately 10% to 15% of boys with ALL. The DFS for patients with occult testicular leukemia treated with testicular irradiation and systemic and CNS chemotherapy is approximately 60% to 70%. The treatment results for occult testicular leukemia and an isolated overt testicular relapse that occurs off therapy are similar. [411] For this reason, documentation of occult disease by performing testicular biopsies in patients on therapy is no longer advocated. [411,679]

### *Other Sites of Relapse*

Leukemia may occasionally recur at other sites (e.g., ovary, eye).[680,681] and [682] If feasible, the appropriate treatment should include local measures for disease control (e.g., radiation therapy) and intensification of systemic chemotherapy.

### Late Effects of Treatment

The improved survival of children with ALL has focused attention on the late effects of antileukemic therapy. A number of adverse sequelae have been identified. These are discussed in more detail in Chapter 49.

CNS sequelae have been particularly concerning. Despite early reports suggesting that CNS preventive therapy with lower dose cranial irradiation and intrathecal chemotherapy was devoid of significant long-term side effects, a large body of evidence indicates that this treatment may produce abnormal CT brain scans (Fig. 19-17), impaired intellectual and psychomotor functions, and neuroendocrine abnormalities. [653,663,683] Four neuropathologically distinct forms of delayed CNS toxicity have been identified in patients with ALL: cortical atrophy, necrotizing leukoencephalopathy, subacute leukoencephalopathy, and mineralizing microangiopathy. [663]

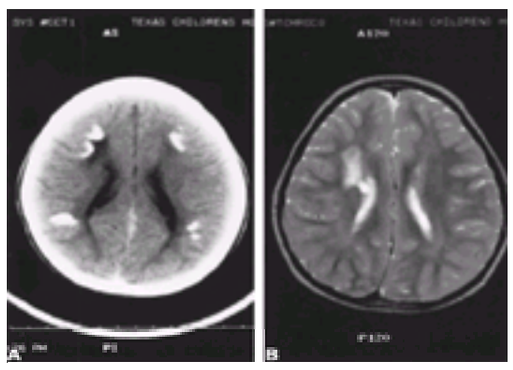

**FIGURE 19-17.** Radiographic measurement of the late effects of chemotherapy and radiotherapy on the central nervous system (CNS). **A:** Computed tomographic image of a patient with acute lymphoblastic leukemia (ALL) and CNS sequelae. Notice the intracerebral calcifications (discrete bright white areas). **B:** Magnetic resonance image of the same patient showing leukoencephalopathy (hypolucent, demyelinated tracts). This patient was originally diagnosed with standard-risk ALL and did not have CNS disease at any time. The patient received standard-risk therapy, which included intrathecal therapy with cytarabine, methotrexate, and hydrocortisone. No radiation therapy was administered. The patient has mild learning and motor disabilities approximately 5 years from diagnosis. Images were obtained several months after completion of therapy.

Cortical atrophy, the most common histopathologic manifestation of CNS treatment, is a well-recognized delayed toxicity of whole brain irradiation. Radiation produces multiple microscopic areas of focal necrosis that eventually cause the loss of cortical tissue and generalized cortical atrophy. [684] Necrotizing leukoencephalopathy, a particularly severe form of delayed neurotoxicity, is relatively uncommon. It occurs most frequently in patients who have received large cumulative doses of cranial irradiation and intrathecal and systemic methotrexate (e.g., for treatment of recurrent meningeal leukemia) but also has been observed in patients who have not received cranial irradiation (Fig. 19-17).[685,686] Leukoencephalopathy is characterized pathologically by multifocal demyelination (Fig. 19-17). Patients with this syndrome may present with a variety of clinical findings, ranging from poor school performance and mild confusion to lethargy, dysarthria, dysphasia, ataxia, spasticity, or progressive dementia.[687,688]

Mineralizing microangiopathy, a degenerative mineralizing disorder of the small vessels, is accompanied by dystrophic calcification of brain tissue, primarily gray matter. It also occurs more frequently in patients who have received greater cumulative doses of cranial irradiation and i.v. methotrexate, particularly in younger children (younger than 6 years). The intracerebral calcifications of mineralizing microangiopathy also can be demonstrated on CT and MRI scans.

The mechanism(s) that underlie the CNS effects associated with cranial irradiation and intrathecal chemotherapy are not entirely clear. Much of the damage may be related to chronic effects on the cerebral vasculature of both CNS irradiation and chemotherapy. [689] Methotrexate and the rises in homocysteine induced by methotrexate have been linked to both acute (seizures) and chronic neurologic toxicity. [689,690]

Numerous studies have demonstrated abnormal CT brain scans in asymptomatic ALL patients who have received CNS preventive therapy, particularly with cranial irradiation and intrathecal chemotherapy. Abnormal CT scan findings identified include ventricular dilatation and widening of the subarachnoid spaces (i.e., cerebral cortical atrophy), decreased attenuation coefficient (i.e., hypodensity of white matter indicating localized edema, demyelination, or both), and intracerebral calcifications (i.e., mineralizing microangiography). [684] The incidence of these abnormalities appears to correlate with the intensity of CNS preventive therapy. [663] Studies have documented a significant association between these abnormalities and neuropsychologic dysfunction. [663,691] The observation that CT scan lesions may first appear as late as 7 to 9 years after initiation of CNS preventive therapy is of concern and emphasizes the importance of long-term follow-up examination. [502,692]

A variety of studies have identified the existence of functional CNS impairment in some survivors of childhood ALL. In addition to significantly impaired academic achievement, problems with poor body image and depression, decreased IQ scores, increased distractibility, and abnormalities in memory and frontal lobe functions have all been documented.[500,663,693,694] Study of interventions for the important psychological and psychosocial consequences of the disease and its treatment for survivors and their families has just begun.[695,696] Careful follow-up with comprehensive neuropsychometric testing may permit early identification of developing deficits and possibly allow therapeutic intervention.

Although new approaches to CNS preventive therapy may offer the possibility of reducing or preventing adverse CNS sequelae, a large number of patients being followed by pediatric oncologists are at risk for developing late CNS toxicity. Diagnostic MRI or CT scans of the brain every 2 to 3 years have been recommended for

patients who have received cranial irradiation. [663,692] Patients who were very young (younger than 8 years) at the time of diagnosis or cranial radiation therapy appear to be at the greatest risk.[663] The finding of an apparent increased incidence of CT and MRI abnormalities in nonirradiated patients treated with intermediate-dose methotrexate and 6-MP indicates the importance of carefully following all patients who have received any form of CNS preventive therapy. [522,697]

Neuroendocrine abnormalities, primarily involving the hypothalamic pituitary axis, also have been documented in children who have received cranial radiotherapy with CNS preventive therapy. The principal finding is decreased growth hormone output measured by response to provocative stimuli or by analysis of basal pulsatile growth hormone secretion.[498,698] The incidence of impaired growth hormone responses to provocative stimuli may be 50% or greater. [699,700] Blunted spontaneous basal pulsatile secretion of growth hormone is a consistent finding in children with ALL who received 2,400 cGy of cranial irradiation and intrathecal methotrexate. [498] Preliminary information suggests that the effect of 1,800 cGy cranial irradiation on pulsatile growth hormone secretion may be less severe. [504] A study found that pulsatile growth hormone output in patients treated with 1,800 cGy, although reduced compared with healthy controls, was not as low as that in patients who had received 2,400 cGy of cranial irradiation. [701]

Short stature occurs in some children with ALL, although there is disagreement about its frequency. [700,702] In many children, “catch-up growth” occurs after discontinuation of therapy; others have persistent short stature. A study of children with ALL treated without cranial irradiation reported that, although an initial decline in height and growth velocity was observed after diagnosis, compensatory increases occur during further treatment. The negative impact of cranial irradiation on growth is well known. Several studies have documented significant reduction in linear growth during and after therapy in patients who received 2,400 cGy or more of cranial irradiation.[703,704] Data demonstrate that the final height of adults who received 2,400 cGy cranial irradiation as children is significantly reduced. [703] Decreases have been reported for patients treated with 1,800 cGy, although this has not been a universal finding. [701,703] The impact of 1,800 cGy on final adult height is reported to be modest, although girls treated with this approach at a young age maybe at risk for significant growth failure. [704,705]

Although abnormal growth hormone output resulting from cranial irradiation may explain the short stature in some children, others have normal hypothalamic-pituitary function, suggesting that growth delay in children with ALL may be multifactorial. [498,706] Effects of therapy on multiple organ systems as well as lung and bone growth itself undoubtedly contribute to the growth problems of some children, both during and after ALL therapy. [707,708 and 709] Nevertheless, the development of short stature after cranial irradiation requires comprehensive endocrine evaluation. If its deficiency is documented, growth hormone replacement may be warranted.

There appears to be a prevalence of obesity among children who have successfully completed therapy for ALL. [706,710] Cranial irradiation is associated with this phenomenon, but it seems likely that corticosteroids and other drugs are contributing factors. One recent study of obesity in ALL patients implicated genetics, finding a substantial number of the patient's mothers were also obese. [711]

Chemotherapy may produce long-term side effects in other organs. Patients receiving maintenance treatment frequently have elevated liver function tests, primarily a reflection of methotrexate hepatotoxicity. After chemotherapy stops, the test results of most patients usually return to normal, and persistent liver function abnormalities are rare.[712] Chronic liver disease is more likely to occur in patients with a history of viral hepatitis and is particularly severe after deltaviral hepatitis infection.[713]

Many current ALL treatment regimens use anthracyclines. Treatment with these agents carries the potential risk of cardiomyopathy, but because the total cumulative doses of these agents in most protocols is considerably lower than 550 mg per $m^2$, clinically significant cardiomyopathy in patients undergoing active treatment has been a relatively rare occurrence. Studies suggest that patients exposed to anthracyclines for treatment of ALL may be at a greater risk for late-onset congestive heart failure than previously appreciated.[714,715 and 716] Exercise or pregnancy may provoke the occurrence of late-onset cardiomyopathy, or it may be spontaneous. In a study from the DFCI, more than one-half of the long-term ALL patients evaluated had evidence of abnormal left ventricular function. [715] Children who are younger at the time of treatment and with a greater cumulative anthracycline dose had a greater incidence of subclinical cardiac damage. This and similar reports have heightened concern about late anthracycline cardiotoxicity and emphasized the importance of careful monitoring and follow-up of children at risk. [714,717] There may be, in fact, no absolutely safe anthracycline dose that will prevent cardiotoxicity. [716,718]

Other chemotherapeutic agents and modalities (i.e., radiation fields that include the cardiac region) may exacerbate anthracycline cardiac toxicity. [719] Studies aimed at reducing and possibly circumventing anthracycline cardiotoxicity by administering these agents by continuous infusion or together with the cardioprotective agent dexrazoxane (Zinecard) are in progress, but follow-up time is too short for analysis (see Chapter 10 and Chapter 49).[720]

Multiple other toxicities are associated with antileukemic agents. Hemorrhagic cystitis and bladder fibrosis from cyclophosphamide treatment, avascular necrosis secondary to steroid treatment, and significant sequelae of L-asparaginase–induced thrombosis and hemorrhagic infarction fortunately are encountered relatively infrequently in patients with ALL. These and other drug-related toxicities are discussed in more detail in Chapter 10 and Chapter 49.

The reproductive capacity and sexual function of patients with ALL treated with prolonged chemotherapy has been studied. [721,722,723 and 724] Primary gonadal damage has been documented in patients of both sexes treated on cyclophosphamide-containing intensive ALL treatment regimens. [725] Cyclophosphamide, ifosfamide, and other alkylating agents continue to be used in the intensification schema and BMT regimens of many groups. Although alkylating agent therapy is known to impair reproductive function, little information exists about the reproductive status of patients treated with other commonly used chemotherapies. In most cases, girls with leukemia retain intact reproductive function. However, the timing of chemotherapy in relation to puberty may be important. [726,727] A study of prepubertal boys who received chemotherapy for ALL, not including cyclophosphamide, also revealed no evidence of significant gonadal dysfunction. [728] Although this evidence is encouraging, additional information on a larger number of patients treated in the periods before, during, and after puberty is required before definitive conclusions about the reproductive capacity of these patients can be drawn.

More information is needed on the outcome of pregnancy after treatment for leukemia. Although our knowledge of the teratogenicity and mutagenicity of antileukemic therapy is incomplete, some data indicate that normal births occur in most cases in which women receive chemotherapy before gestation or after the first trimester. [729] Chemotherapy administered to men close to or before the time of insemination does not appear to result in fetal damage. Although available information suggests reason for cautious optimism, additional long-term follow-up of the offspring of survivors of childhood leukemia is needed.

The risk of second malignant neoplasm (SMN) in children with ALL has been estimated to be between 3% and 12% in the 5 to 24 years after primary diagnosis. [730,731 and 732] This represents a six- to tenfold increase in risk, compared to the general population, for the development of future tumors. The nonhematopoietic SMNs tend to occur 5 to 10 years after the original ALL, and in many cases occur within or in close proximity to the radiation fields used in the original therapy. [733,734] Although a causal relation between the development of secondary brain tumors and cranial irradiation is likely, CNS tumors have also been reported in a small number of patients who have not received cranial irradiation. [735]

Investigators at SJCRH reported an unexpected high incidence of secondary AML among a large group of patients treated with intensive chemotherapy, particularly those with T-cell ALL.[736] These leukemias developed a median of 3 years after the diagnosis of ALL and were predominantly characterized by an 11q23 chromosomal abnormality. The risk of developing secondary AML within 6 years of achieving initial remission in this study was estimated to be 5%. There appears to be a strong relation between exposure to the epipodophyllotoxins and other topoisomerase II inhibitors, anthracyclines, and actinomycin D and the development of secondary AML with 11q23 abnormalities.[736] Similar secondary leukemias have been observed in other cancer patients treated with these drugs. [737,738] Concern over these secondary myeloid leukemias has led to a reappraisal of the role of the epipodophyllotoxins in the treatment of ALL. [739,740]

Despite the degree of increase in risk of SMNs, the risk of other first events (i.e., induction failure, relapse, or death from another cause) remains tenfold higher than the risk of developing a SMN (Fig. 19-18).[733] Most reported SMNs are brain tumors (gliomas of varying histologic grades) in patients who have had radiation therapy and hematopoietic tumors (primarily AML or MDS) in all patients.[732,733] Other reported tumors include thyroid tumors, melanoma, dysgerminoma, ganglioneuroblastoma, leiomyosarcoma, mucoepidermoid carcinoma of the parotid, osteosarcoma, adenocarcinoma of the colon, and testicular carcinoma. [732,733,741,742]

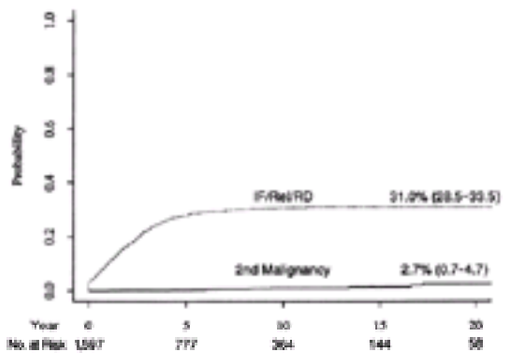


**FIGURE 19-18.** Risk of induction failure (IF), relapse (Rel), and remission death (RD) far exceeds the risk of second malignancies in patients treated for acute lymphoblastic leukemia. (From Kimball Dalton VM, Gelber RD, Li F, et al. Second malignancies in patients treated for childhood acute lymphoblastic leukemia. J Clin Oncol 1998;16:2848–2853, with permission.)

Physicians should be aware of the effects of previous chemotherapy on the immune system of children treated for ALL. Earlier studies suggested that recovery of the immune system occurs within the first year after completion of chemotherapy. More recent studies show that a significant percentage of patients have abnormally low immunoglobulins and other evidence of immune suppression as late as 2 years after completion of treatment.[743] As many as one-third of patients have low antibody titers to clinically significant viruses to which they had been previously immunized.

The psychosocial status of long-term survivors of ALL is an area of considerable concern. Compared with matched groups of controls or with siblings, survivors of ALL are likely to have more behavioral problems, lower levels of life satisfaction, impaired attainment of social skills, and poorer school performance.[488,694,744] Some of these problems may be preventable through modification of treatment (e.g., alternative methods of CNS preventive therapy). Despite the fact that many children with ALL have tolerated their treatment well and may not experience significant late sequelae, the medical community must be aware of the special problems and needs that children with ALL may incur as they are reincorporated into mainstream society. Misapprehension and fears about cancer in general and leukemia in particular are still widespread, and acceptance of patients into normal community activities is not always enthusiastic.

## FUTURE CHALLENGES

Although the scientific and technologic future appears bright and the cure rate for children with ALL continues to climb, the disease continues to present a significant challenge. At present, 20% to 25% of patients are dying from their disease, and significant numbers of the survivors are likely to emerge from current therapies with adverse physical and psychosocial sequelae.

Formidable obstacles remain before cure becomes a reality for all children. Laboratory studies and future clinical trials must focus on resolving a number of critical issues. These include improving therapy for patients at high risk for relapse, developing a better understanding of the biologic causes of ALL, refining methods of identifying patients at risk for relapse, understanding the causes of treatment failure, and overcoming both acute and long-term toxicities of current treatment. In addition, more effective treatment must be found for patients who relapse from conventional chemotherapy.

Improving therapy for patients at high risk for relapse is perhaps the greatest challenge. The recent strategy for approaching such patients has been to significantly intensify treatment. Although this approach has been moderately successful and has improved the cure rate, the associated toxicities are of concern and make further intensification improbable given current supportive care techniques.

Maximal intensification can be achieved through BMT. Indeed, BMT with conventional and alternative donor sources continues to show promise for selected groups of patients. The apparent successes using related, partially matched, and matched unrelated donors has increased the likelihood that the constraints of the HLA barrier may eventually be successfully circumvented, making marrow transplantation an option for an increasing number of high-risk patients. Although the results of autologous transplantation have been disappointing to date, novel antibody and molecular-based purging techniques continue to be studied and a role for autologous transplantation in ALL treatment may eventually prove promising.

Perhaps the most exciting approach to prevention of relapse lies in the use of novel tumor vaccines that are designed to prevent relapse by stimulating the host immune response to leukemia cells.[745,746 and 747] This is being actively studied in several centers.

In the search for effective new treatments, the effort to develop new molecularly targeted therapies is perhaps the most exciting area of research (see below). At present, there are a large number of promising agents in various stages of clinical study (see Chapter 14). These include agents such as tyrosine kinase, farnesyl transferase, purine pathway (e.g., compound 506U), and angiogenesis inhibitors, as well as differentiating agents and other novel biologic compounds.

In the future, the most effective new therapies will evolve from a better understanding of the biology of ALL. The recent sequencing of the human genome now places us in a position to understand leukemia biology at a level heretofore unimagined. Work has already begun to expand and refine our knowledge of leukemia biology using techniques derived from the Human Genome Project and the virtual revolution in computer sciences of the past several years, such as cDNA microarray, SKY, and other recent advances in computational biology. By allowing sophisticated comparisons between previously unimagined amounts of laboratory and clinical data, it is anticipated that the application of these new technologies will improve diagnostic methodologies and refine prognostic stratification.

Ultimately, it is hoped that these technological advances will provide new, more targeted and potentially less toxic treatment approaches. In addition, pharmacogenomics promises to revolutionize chemotherapy by individualizing both the design and dosing of ALL treatment regimens. However, the role of new approaches to therapy will require confirmation in organized, carefully conducted clinical trials. Furthermore, as in the past, childhood ALL may well provide a compelling paradigm for the successful application of the new technologies to cancer treatment.

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# ACUTE MYELOGENOUS LEUKEMIA

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## INTRODUCTION

Acute myelogenous leukemia (AML) represents a heterogeneous group of hematologic malignancies that arise within bone marrow precursors of the myeloid, monocyte, erythroid, and megakaryocytic cell lineages. Although AML comprises only approximately 15% to 25% of childhood leukemia, it still accounts for greater than 30% of deaths from leukemia.

Despite steady improvement in the treatment of children with AML over the past three decades, event-free survival (EFS) continues to be less than 50% in most large series. The principal reasons for treatment failure include (a) the development of resistance to multiple chemotherapeutic drugs and (b) treatment-related mortality. Resistance to therapy may be present at the time of diagnosis or arise during treatment or at the time of disease relapse. Although many advances have been made in the identification of resistance pathways and in attempts in ongoing clinical trials to circumvent tumor cell resistance to chemotherapeutic agents, it is still not possible to overcome the problem of drug resistance in most patients with AML. Furthermore, the morbidity and mortality associated with currently used chemotherapy significantly limit its success. A major challenge for the future will be to overcome drug resistance of the leukemic blasts while reducing the short- and long-term side effects of treatment. An improved understanding of the molecular heterogeneity of AML should provide important clues to successfully meeting this challenge.

## EPIDEMIOLOGY

Approximately 3,500 children per year develop acute leukemia in the United States. [1] AML represents approximately 15% to 25% of this number for a total of approximately 500 children per year. Although AML remains the most frequently encountered acute leukemia in adults, the ratio of AML to acute lymphocytic leukemia (ALL) in children remains approximately 1:4, except in the neonatal period in which there is a peak in the incidence of AML. [1] The frequency of AML remains stable throughout childhood, but shows a slight increase during adolescence. During adulthood, and particularly beyond age 55 years, there is a dramatic increase in the incidence of AML, much of which is a result of secondary AML and the rise in myelodysplastic syndromes (MDSs). [1] There is no difference in the incidence of AML between males and females. Surveillance, Epidemiology, and End Results Program data from the National Cancer Institute show no significant changes in the incidence of childhood AML over the past two decades. [1]

There is evidence for variation in the incidence of AML among some racial and ethnic groups. For example, black children have an incidence of 5.8 cases per million compared to 4.8 cases per million in white children. [2,3] Children of Hispanic background have the highest incidence. [4,5,6] and [7] Most subtypes of AML are distributed equally in all ethnic and racial groups, with the exception of acute promyelocytic leukemia (APL), which appears to have a higher incidence in the Hispanic and Latin populations. There is a higher incidence of AML in Asia. [4] Of growing concern in the pediatric population is the incidence of secondary leukemia, resulting from chemotherapy and radiation treatment for other malignancies. [8,9,10,11,12] and [13]

## PREDISPOSING FACTORS

### Environmental Risks

Risk factors associated with the development of AML can be either environmental or secondary to inherited or acquired predisposing conditions ( Table 20-1). Significant exposure to ionizing irradiation results in a 10- to 20-fold increase in the incidence of AML. For example, individuals who were exposed to radiation from the atomic bombs dropped on Nagasaki and Hiroshima during World War II developed a 20-fold increase in AML and chronic myelogenous leukemia (CML). [14,15,16] and [17] The incidence peaked between 6 to 8 years after the exposure, but remained significantly higher over the next 20 years when compared to unexposed individuals. [14,15,16] and [17] No increase in leukemia was observed in children exposed prenatally to the ionizing radiation from these atomic bombs. [18] Whether there is an increased incidence of leukemia in children prenatally exposed to diagnostic x-rays remains controversial. [19] There is also no convincing evidence that prenatal or postnatal exposure to ultrasound or the effects of electrical power lines increases the risk of AML. [19,20,21,22,23,24,25] and [26]

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|---|
| **Environmental** |
| Ionizing radiation |
| Chemical exposures |
| **Pesticides** |
| **Petroleum products** |
| **Cytotoxic chemotherapy (alkylating agents, epipodophyllotoxins)** |
| **Prenatal alcohol exposure of fetus** |
| **Prenatal marijuana (?tobacco) exposure of fetus** |
| **Inherited** |
| **Twinning** |
| **Down syndrome** |
| **Fanconi's anemia** |
| **Kostmann's syndrome** |
| **Shwachman-Diamond syndrome** |
| **Diamond-Blackfan syndrome** |
| **Neurofibromatosis type 1** |
| **Ataxia telangiectasia** |
| **Klinefelter's syndrome** |
| **Li-Fraumeni syndrome** |
| **Bloom syndrome** |
| **NonInherited** |
| **Aplastic anemia** |
| **Myelodysplastic syndrome** |
| **Paroxysmal nocturnal hemoglobinuria** |

**TABLE 20-1. PREDISPOSING CONDITIONS**

The exposure to environmental chemical toxins and increased risk for leukemia has been an area of immense interest concerning the development of AML. Prenatal exposure to maternal cigarette smoking has been associated with an increased risk of developing AML.[3,27,28,29] and [30] Maternal use of marijuana and consequential prenatal exposure has also been linked in some studies to the development of AML in exposed offspring, but subsequent studies have not confirmed this finding.[3,7,31] Maternal alcohol usage also has been reported to be associated with the subsequent development of leukemia in offspring.[28,29] A variety of chemical exposures, including petroleum products, benzene, herbicides, and pesticides, have been closely linked to the development of MDS and AML.[32,33,34] and [35] Although exposure to such chemicals has been eliminated in schools and in many workplaces, the risk to some individuals, such as farmers and migrant workers, continues to be problematic.[36]

A growing area of concern that is, in part, a result of the success of cancer treatments, involves the increased incidence of secondary leukemia after treatment of primary malignant as well as nonmalignant conditions.[10,37] Such exposures take on increased importance for children, adolescents, and young adults who will, by virtue of their age, have prolonged periods of risk. For example, treatment of patients with alkylating agents, such as cyclophosphamide, ifosfamide, nitrogen mustard, chlorambucil, and melphalan, is linked to an increased incidence of MDS and AML, with a peak incidence at 4 to 5 years after initial treatment, but with some cases still occurring 10 to 12 years later.[9,38,39] and [40] The leukemogenic effect of epipodophyllotoxins, such as etoposide (VP-16), is now well established.[39,41] The first reports on the association of prolonged exposure to etoposide for the development of AML came out of clinical trials in children with ALL.[11,13,42] There is no convincing epidemiologic or laboratory data that there is a viral etiology for AML.

## Genetic Risks

Most cases of AML arise in patients for whom no known genetic predisposition is known. Most patients have neither a family history of cancer predisposition nor clinical features, such as developmental abnormalities, that would suggest predisposing inherited risk factors for the development of AML. It remains to be determined, however, whether such “sporadic” cases of AML indeed arise without predisposition, or whether these cases arise in patients with as yet unrecognized, more subtle leukemia-promoting genetic backgrounds. Less commonly, clinically evident genetic risk factors for AML are evident, as detailed in the following sections.

### *Twins*

Several genetic risk factors have been identified that predispose individuals to develop AML. These factors may be inherited or acquired. The increased frequency of leukemia (both AML and ALL) in siblings of patients with leukemia as well as the relatively rare occurrences of familial leukemia strongly suggest an important hereditary contribution in certain instances.[43,44,45] and [46] For nonidentical twins, an estimated two- to fourfold increase for developing leukemia has been made. The risk decreases with age, so that beyond approximately 6 years of age the risk is not significantly greater than that of the general population. The high concordance of AML in identical twins also argues in part for an important hereditary contribution.[47,48] When one identical twin develops leukemia before the age of 6 years, the risk of the other twin developing leukemia is approximately 20% to 25%.[49] A part of the high concordance during the first year of life is due to the transplacental transfer of leukemia cells between twins, in which case, the leukemia usually arises with weeks to a few months in the second twin.[50,51,52] and [53]

### *Down Syndrome*

Although children with Down syndrome (DS) represent the most common inherited condition predisposing to the development of leukemia, it remains unclear what specific gene(s) on chromosome 21 is the cause of this predisposition.[54,55,56] and [57] A number of candidate genes, such as the AML1 gene, have been implicated because of their chromosomal location, but no definitive evidence has been obtained proving mutations of a single gene or true dosage effect contributing to the increased incidence of leukemia in these patients.[57,58] Nevertheless, children with DS have an approximately 14-fold increase over the general population for developing leukemia.[54,55,56] and [57] Of further interest is that although children with DS have a similar frequency of ALL and AML in later childhood, the incidence of AML predominates during the first 3 years of life, and the development of megakaryoblastic leukemia is more common than other subtypes.[59,60] and [61] Patients with DS also have an increased predisposition to develop a transient myeloproliferative disorder (TMD), that, though clinically indistinguishable from congenital leukemia, usually is self-resolving. This syndrome is further discussed later in this chapter. The close association of trisomy 21 with TMD further supports the role that the genes involved in the development of AML in patients with DS are likely to be involved in the control of normal myelopoiesis.

### *DNA Repair and Leukemogenic Risk*

An increased frequency of AML has also been strongly linked to a number of inherited disorders due to defects in genes regulating cell-cycle progression as well as DNA repair.[62,63] For example, patients with Fanconi's anemia, an autosomal inherited disorder, are particularly prone to develop MDS and AML.[64,65] and [66] It has been demonstrated that the incidence of AML in patients with Fanconi's anemia is more than 15,000 times that observed in children in the general population.[65,66] The actuarial risk of MDS or AML has been shown to be approximately 52% by 40 years of age.[67] Although the genetic defect in patients with Fanconi's anemia may represent different genes that functionally are related to one another, the end cellular defect results in alterations in the $G_2$ stage of the cell cycle along with a predisposition for chromosomal instability and a hypersensitivity to DNA damaging agents.[68,69] and [70] Children with Bloom syndrome, another autosomal inherited disorder, show a variety of developmental abnormalities in terms of skeletal and immune function, but also show a profound defect in resolving chromosomal recombination events, leading to a high frequency of abnormal chromosomal exchanges.[68,69] and [70] Bloom syndrome is now known to be due to the inheritance of a defective helicase, BLM, involved in DNA recombination.[71,72] and [73] Patients with ataxia telangiectasia, another autosomal inherited disorder resulting in neuromuscular deterioration and immune deficiency, also show a high frequency of developing leukemia.[72] The mutant gene product, ATM, that causes this syndrome, has been identified and shown to play a role in DNA sensitivity to genotoxic agents.[72,74]

### *Growth and Apoptosis Signaling Pathways*

Patients with neurofibromatosis type 1 (NF-1) represent an alternative inherited molecular pathway leading to a predisposition to develop AML.[75,76] A defective neurofibromin gene product is the cause of this disorder. Neurofibromin is a GTPase that is integrally involved in inactivating the proto-oncogene RAS by converting it from an active deoxyguanosine triphosphate (GTP) state to an inactive guanosine diphosphate (GDP) state.[77] Thus, patients with NF-1 have increased levels of activated RAS, which results in dysregulated cell proliferation and survival, that is clinically manifested by the development of neurofibromas and an increased incidence of AML. Recent data have indicated that patients with severe congenital neutropenia (i.e., Kostmann's syndrome) have an increased risk of developing MDS and AML, and that the risk increases with age.[78,79] The introduction of granulocyte colony-stimulating factor (G-CSF) for the treatment of these patients has clearly allowed more patients to live longer so that it is unclear whether the development of AML is due to the intrinsic disorder or a combination of an inherited predisposition plus G-CSF exposure. The discovery that a significant number of patients with Kostmann's syndrome have functional mutations in the gene encoding elastase has suggested that such proteases may affect other proteins involved in cell growth and apoptosis.[80] In addition, the development of activating mutations of the G-CSF receptor have been observed during the development of AML in these patients, but such mutations appear to be an acquired and relatively later genetic

event.[81,82]

### Acquired Predisposition

Acquired conditions can also predispose to the development of AML. For example, as many as 20% of patients with aplastic anemia treated with immunosuppressive regimens may eventually develop MDS or AML, or both.[83,84] At this time, it remains unclear what the contribution is from an intrinsic genetic alteration of a myeloid precursor or from the immunosuppressive agents. Another acquired disorder of hematopoietic precursors, paroxysmal nocturnal hemoglobinuria, is associated with an increased frequency of AML. The development of MDS should also be considered a predisposing condition for AML. In this context, the acquisition of certain somatic chromosomal abnormalities, often associated with MDS, may predispose individuals to develop AML. [85,86] For example, the development of monosomy 7 in bone marrow precursors, may be associated with an increased frequency of disorders of the myeloid lineage, including MDS and AML. [87,88] The acquisition of a variety of other genetic alterations, most commonly translocations, involving genes that regulate cell growth, differentiation, and apoptosis, also plays a fundamental role in the etiology of AML.[86,89]

Similar to genetic models that have been proposed to explain the development of colon cancer as well as solid tumors associated with inherited defects the retinoblastoma or p53 genes, the development of AML is the result of an accumulation of several genetic abnormalities. [90] The inheritance of predisposing genetic defects is likely to result in the earlier acquisition of other mutated genes involved in cell survival, chromosomal stability, or DNA repair and result in an increased frequency of AML at an earlier age.[89] The accumulation of genetic mutations over the course of a lifetime contributes to the increased frequency of AML during later adulthood. In addition, the types of genetic defects that occur can influence whether the leukemia develops characteristics representative of one of a variety of myeloid lineage phenotypes.

## BIOLOGY AND HIERARCHY OF THE ACUTE MYELOGENOUS LEUKEMIA STEM CELL

AML develops as a result of genetic changes that occur in primitive hematopoietic stem cells, resulting in the expansion of leukemic cells often displaying an incomplete block in normal differentiation. [91,92,93] and [94] Thus, leukemic cells often display many of the features observed in their normal hematopoietic counterparts such as similar morphology, cytoplasmic enzyme synthesis, surface markers, dependence on cytokines for survival, and the ability to generate more mature cells from less mature precursors.[92,95,96] The heterogeneity of these malignant myeloid disorders may arise from the types of genetic changes that have occurred as well as what stage of differentiation the leukemic stem cell becomes transformed. [97]

There is accumulating evidence that the leukemic stem cell in different subtypes of AML and MDS arises at various stages of differentiation as defined by surface marker characteristics as well as *in vitro* leukemic colony assays and *in vivo* leukemia, initiating measurements utilizing transplantation into immunodeficient mice. [91,92] It is generally considered that leukemias with different degrees of differentiation result from oncogenic transformation of hematopoietic progenitors of different states of maturation. That is, it is the cell of transformation that dictates the phenotype of the leukemia. An alternative, equally plausible hypothesis is that all types of AML occur through transformation of very early, stem cell–like hematopoietic cells. In that scenario, it is the transforming oncogene, not the cell of origin, which determines the degree of differentiation observed in the leukemia.

Several strategies have been used to define the molecular changes that are involved in the conversion of a normal hematopoietic progenitor to a malignant phenotype. Cytogenetic analysis with standard, and now more sophisticated methods of fluorescent *in situ* hybridization (FISH), have helped to identify critical chromosomal regions and have been instrumental in the identification of important genes regulating myeloid cell growth, differentiation, apoptosis, and immune recognition, as well as gene products involved in DNA repair and chemotherapeutic resistance. [93]

Initial studies by Fialkow and colleagues using markers on the X-chromosome and the presence of the Philadelphia chromosome demonstrated the heterogeneous involvement of the hematopoietic stem cell and myeloid precursor cell compartments by different forms of myeloid leukemia. [92,98] These studies have been extended by many subsequent investigators to show a complex pattern of involvement of myeloid precursor compartments by AML stem cells. Using flow cytometric analysis and sorting, various precursor cell compartments can be isolated on the basis of specific surface antigen expression patterns. [99] For example, some studies have shown that translocations or chromosomal abnormalities such as t(8;21) or inv(16) are found in the CD33–, CD38–, CD34+, primitive hematopoietic precursor compartment.[91,92,99] Other investigations, using fluorescent activated flow sorting to sort hematopoietic cellular compartments and FISH analysis to detect specific chromosomal abnormalities, have shown that the primary cytogenetic change in many cases of AML is present in the CD34+ primitive stem cell compartment. In contrast, the t(15;17) translocation associated with APL is detected in precursors at a later stage of differentiation than the primitive stem cell. [91,92,99]

These studies have important clinical implications. If the leukemic stem cell arises within a very primitive hematopoietic compartment, then the biologic proximity and similarity to the normal hematopoietic stem cell may be quite high. This would result in possibly fewer biologically important differences to be therapeutically exploited to eradicate the leukemic stem cell while sparing the normal hematopoietic stem cell. If the leukemic stem cell develops in a more differentiated lineage compartment, such as may be the case in promyelocytic leukemia, then therapeutic strategies may be able to exploit a wider range of differences that distinguish leukemic and normal stem cells (Fig. 20-1). In addition, the heterogeneity of AML as a disease may provide the means to direct therapies to different subtypes as defined by a variety of criteria, potentially ranging from morphology to genetic expression patterns. Thus, understanding the different types of AML and their genetic origin is fundamental to the development of more effective therapies.

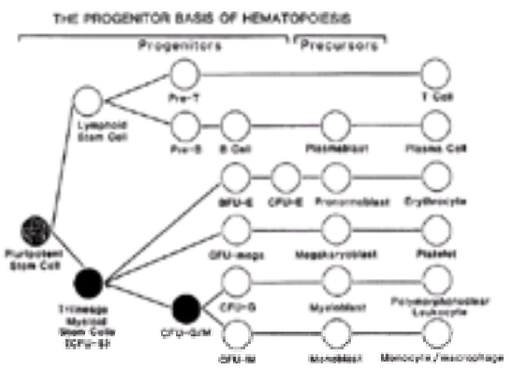


**FIGURE 20-1.** Stem cell diagram showing sites of malignant transformation in acute myelogenous leukemia (*shaded*) and chronic myeloid leukemia (*stippled*). Colony-forming units (CFUs) are single cells that can form colonies when bone marrow is cultured *in vitro*. BFU-E, bone marrow stem cell assay.

## CLASSIFICATION OF ACUTE MYELOGENOUS LEUKEMIA: AN EVOLVING PROCESS

A good disease classification schema is one that provides a better understanding of the disorder and helps to direct and improve the outcome of treatment as well as serve as the basis for more useful, subsequent classifications. Although the first published cases of leukemia occurred independently in the mid-1800s by Bennett and Virchow, it was not until the latter part of that century, with the further refinement of staining methods, that a distinction between myeloid (derived from bone marrow) and lymphoid (derived from lymphatic tissues) acute leukemias was defined. [100] With increased spread of the microscope and improved histochemical stains, the age of morphologic classification blossomed in the early and middle part of the twentieth century, eventually resulting in a morphologic schema for classifying AML. This effort culminated in the French-American-British (FAB) classification schema first established in 1976 and revised in 1985. [101,102] This system is primarily based on morphologic criteria after staining with Wright, Wright-Giemsa, or May-Grunwald stain, in addition to some histochemical-defined markers ( Table 20-2).

| FAB type | Name | Morphology | Histochemistry |
|---|---|---|---|
| M0 | Acute myeloblastic: no maturation | Undifferentiated; >90% blasts; <10% promyelocytes/monocytes | MPO+; SBB+; PAS- |
| M1 | Acute myeloblastic with maturation | ≥90% and ≤95% blasts; >10% promyelocytes/myelocytes; ≤20% monocytic cells | MPO+; SBB+; PAS- |
| M2 | Acute promyelocytic: hypergranular type | >20% abnormal hypergranular promyelocytes; Auer rods common | MPO+; SBB+; PAS- |
| M2v | Acute promyelocytic: microgranular variant | Fine granularity of cytoplasm in promyelocytes; folded nuclei | MPO+; SBB+; PAS- |
| M4 | Acute myelomonocytic | ≥20% blasts in nonerythroid series; >20% but <80% monocytic cells; blood monocytes >5 × 10⁹/L; lysozyme >3× normal | MPO+; NASDA+ |
| M4Eo | Acute myelomonocytic with eosinophilia | >5% abnormal eosinophils with basophilic granules | MPO+; NASDA+ eosinophils; AAN+ |
| M5a | Acute monocytic | >80% of monocytic cells = monoblasts; rest are promonocytes/monocytes | MPO+; NASDA+ |
| M5b | Acute monocytic with differentiation | <80% of monocytic cells are monoblasts; rest are promonocytes/monocytes | MPO+; NASDA+ |
| M6 | Acute erythroleukemia | >50% of nonerythroid cells are blasts; <90% of marrow are erythroblasts | PAS+; ringed sideroblasts with iron stain |
| M7 | Acute megakaryoblastic | >50% of nonerythroid cells are megakaryoblasts; cytoplasmic blebs; myelofibrosis common | MPO-; SBB-; NASDA+; platelet MPO+ by EM |

EM, electron microscopy; MPO, myeloperoxidase; NASDA, naphthol-AS chloroacetate; NSE, nonspecific esterase; PAS, periodic acid-Schiff; SBB, Sudan black B

**TABLE 20-2. FRENCH-AMERICAN-BRITISH (FAB) CLASSIFICATION OF ACUTE MYELOGENOUS LEUKEMIA**

## Morphologic and Histochemical Classification: The Beginning of Subtypes

The FAB classification system includes seven subtypes (M1 to M7), each characterized by specific morphologic, histochemical, and, more recently, immunophenotypic and cytogenetic features. The M0 subtype has been used to describe acute undifferentiated leukemia. [103] All subtypes are represented in children and adolescents with AML. Although the original classification schema required 30% myeloblasts in the bone marrow for the diagnosis of AML, the new World Health Organization (WHO) classification accepts the presence of 20% or more bone marrow myeloblasts to be sufficient for diagnosis of AML. In addition, the WHO classification proposal defines subsets of AML based on morphologic and cytogenetic characteristics. [104,105,106,107] and [108] However, such numbers may be quite artificial when one considers the pathophysiologic characteristics of AML. Probably more important than an absolute number of blasts is the progression of accumulation of leukemic blasts with replacement of normal bone marrow elements. Additional criteria have been established that define the different subtypes according to the percentage of myeloblasts with specific lineage characteristics, such as monoblasts or promyeloblasts.

Morphologic classification, along with the most commonly used histochemical stains, is usually sufficient to distinguish AML and its subtypes from ALL ( Fig. 20-2). The peroxidase reaction [i.e., staining for myeloperoxidase (MPO) activity] is present in the primary (azurophilic) granules of both myeloid and monocytic precursors. Auer rods are needle-shaped accumulations of primary granules and are commonly observed in the M2, M3, and M5 subtypes of AML. Positive staining with Sudan black B (SBB) detects intracellular lipids that are found in secondary (basophilic) granules of both myeloid and monocytic precursors. Detection of nonspecific esterase (NSE) is quite characteristically observed in monocytic cells, although weak staining can often be observed in myeloid precursors. Staining for the presence of chloroacetate esterase is relatively specific for cells of the granulocytic lineage. NSE staining will usually be able to distinguish M1 to M3 subtypes from M4 or M5. The M5 subtype is always NSE positive, regardless of whether the MPO reaction is positive or negative. M7 AML may also be positive for NSE. The periodic acid-Schiff (PAS) reaction and staining for the enzyme terminal deoxynucleotide transferase are usually negative in AML, except in the case of the erythroleukemic subtype, M6, which is positive for PAS staining. Detection of platelet peroxidase activity is characteristic of megakaryoblastic leukemia or M7 subtype. The judicious use of morphology and histochemical staining can usually distinguish the various subtypes of AML.

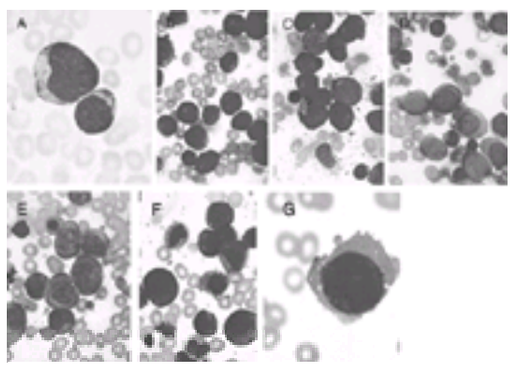


**FIGURE 20-2.** Morphologic French-American-British subtypes of acute myelogenous leukemia: **(A)** M1, **(B)** M2, **(C)** M3, **(D)** M4, **(E)** M5, **(F)** M6, **(G)** M7. May-Grunwald staining was used in all frames. (See Color Figure 20-2.)

## Immunophenotyping: Lineage in Greater Detail

The role of immunophenotyping has taken on an increasingly important role in the diagnosis of leukemia. This approach takes advantage of the development of monoclonal antibodies (MABs) to specific cell surface antigens that are differentially expressed during hematopoietic differentiation. The antigens are usually referred to as *antibody cluster determinants* or *CD* followed by an assigned number, such as CD1, CD2, and so forth. The detection of specific cell surface expression of these lineage- and stage-specific proteins by fluorescence-activated flow cytometry allows a rapid and accurate refinement to the subclassification of the acute leukemias. The specificity and sensitivity of distinguishing AML from ALL by cell surface immunophenotyping are very high. [109,110,111] and [112] In addition, because leukemia cells often display a degree of abnormal expression patterns of specific cell surface antigens, they can be distinguished from normal hematopoietic precursors. [113] For example, aberrant antigen expression has been described to take on one of at least four patterns. These include

1. Coexpression of nonmyeloid antigens
2. Asynchronous expression of antigens such as when an early lineage marker, such as CD34, is coexpressed with a much later antigen, such as CD15
3. Increased expression of a particular antigen significantly above normal levels observed in normal progenitors
4. The absence of myeloid antigens

This abnormal expression pattern can thus be used for the detection of minimal residual disease (MRD) (see under Response to Therapy and Minimal Residual Disease). [113,114] and [115]

Cell surface proteins with particular utility in the diagnosis of AML include CD11b, CD13, CD14, CD15, CD33, CD34, CD41, CD42, and CD61, glycophorin A, class II human leukocyte antigens (HLA-DR), and the stem cell factor receptor, c-Kit. [116,117] During normal hematopoietic maturation, early myeloid precursors are characterized by the expression of CD13, CD33 and CD34. As differentiation proceeds, CD34 expression is lost, CD33 expression decreases, and CD15 becomes expressed. HLA-DR becomes expressed as myeloid differentiation proceeds. When cells differentiate along the monocytic lineage, the expression of such markers as CD14 is often observed, thus making this antigen especially useful in the identification of acute myelomonocytic (M4) and monocytic (M5) leukemia. CD36 and CD64 may also be used to define cells with monocytic differentiation. Progenitors that differentiate along the erythroid lineage express glycophorin A, which helps define acute erythroblastic leukemia (M6). Differentiation along the megakaryocytic lineage is accompanied by expression of platelet-associated proteins, such as CD41, CD42, and CD61, thereby providing a means of identifying acute megakaryocytic leukemia (M7). CD36 is also observed on megakaryocytes. However, it is important to keep in mind that false-positive results have been obtained for cell surface platelet antigens as a result of platelets becoming stuck to the surface of blasts of a non-M7 type of leukemia.[118] When there is any question, the detection of intracytoplasmic platelet proteins, such as platelet peroxidase, should be definitive.

The pattern of expression of such cell surface markers, along with those characteristic of the lymphoid lineage, is informative in terms of distinguishing AML from ALL in greater than 90% of cases (Table 20-3). [109,110,111] and [112] However, leukemia cells, like other types of cancer, may show abnormal expression of proteins not restricted to their own lineage (“lineage infidelity”). For example, proteins, traditionally believed to be expressed on only lymphoid lineage cells, may be expressed on AML blasts in up to 60% of cases. [119,120] Myeloid-associated antigens may also be expressed on ALL blasts. [119,121]

| Marker | M0 | M1 | M2 | M3 | M4 | M5 | M6 | M7 |
|---|---|---|---|---|---|---|---|---|
| **Myeloid** | | | | | | | | |
| CD13 | ++ | + | + | + | ++ | ++ | + | + |
| CD14 | – | – | – | – | ++ | ++ | – | – |
| CD15 | + | + | + | ++ | ++ | ++ | – | – |
| CD33 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| CD34 | ++ | ++ | ++ | + | + | + | + | + |
| CD65 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + |
| **Erythroid** | | | | | | | | |
| Glycophorin A | – | – | – | – | – | – | ++ | – |
| **Megakaryocytic** | | | | | | | | |
| CD41/61 | + | – | – | – | – | – | – | ++ |
| CD42 | + | – | – | – | – | – | – | ++ |
| CD36 | + | + | + | | ++ | ++ | | ++ |
| **B lineage** | | | | | | | | |
| CD10 | – | – | – | – | – | – | – | – |
| CD19 | + | – | – | – | – | – | – | – |
| CD20 | – | – | – | – | – | – | – | – |
| **T-cell lineage** | | | | | | | | |
| CD7 | + | + | + | + | + | + | – | ++ |
| CD3 | – | – | – | – | – | – | – | – |
| CD4 | + | – | – | | ++ | ++ | – | + |
| CD7 | + | + | + | + | + | + | + | + |
| CD56 | + | + | + | + | + | + | – | + |
| **Other** | | | | | | | | |
| HLA-DR | ++ | ++ | ++ | + | +++ | +++ | – | + |
| CD117 | ++ | ++ | + | + | + | +/- | + | + |

+, minimal expression; ++, moderate expression; +++, high expression; +, variable expression; –, no expression.
[a]CD13 expression has been reported to be present on 100% of M5a but negative on M5b.[11]

**TABLE 20-3. IMMUNOPHENOTYPING OF ACUTE MYELOGENOUS LEUKEMIA**

An important question that arises with such cases is how specific markers are (i.e., does apparent lineage infidelity in fact represent lineage fidelity?). Part of the answer is clearly that what was believed to be lineage restricted simply is not. For example, many markers originally thought to be characteristic of the lymphoid lineage can also be expressed in AML. For example, historically, markers such as CD7 and CD4 were considered to be T-cell–specific antigens, though the former is now known to be expressed on progenitor cells and the latter on monocytes. Not surprisingly, these markers are frequently found in AML. Similarly, markers believed to be B-lineage specific (e.g., CD10, CD19, and CD24) or the natural killer cell marker, CD56, can also be found in AML, more frequently in M4 and M5 subtypes.[122,123 and 124] Myeloid-associated antigens may also be expressed on ALL blasts.[119,121] Furthermore, some expression of CD2 has been found on nearly all of AML M3 subtypes (particularly positive in the microgranular type) and in many cases of M4Eo.[125,126,127 and 128] CD4, originally believed to be characteristic of T-helper lymphocytes, is also expressed on cells of the monocytic lineage and monocytic leukemia.[129] Such examples may suggest that the stage of leukemic cell transformation may be normally represented in extremely low frequency rather than an abnormal phenotype. This possibility may imply that immunophenotyping as a method for detecting MRD is compromised. However, depending on the sensitivity that is required for predicting outcome with MRD, this concern may not be practical in that extremely low frequency events (i.e., a normal precursor with coexpression of myeloid and lymphoid markers) would not be routinely detected.

There has therefore not been a consensus on how best to define biphenotypic or mixed lineage leukemia, although most laboratories require more than the coexpression of a single surface marker of a different lineage on an otherwise typical case of AML or ALL. Usually more than one marker from the opposite lineage is required to be expressed on the same leukemic blast to call an acute leukemia biphenotypic. Several “scoring” systems have been proposed that include morphology, histochemistry, immunophenotype, and even gene rearrangements (e.g., T-cell receptor or immunoglobulin gene rearrangements).[130] However, the coexpression of these markers has not been definitively shown to have significant diagnostic or prognostic significance, although treatment in cases of true biphenotypic leukemia is often directed to the dominant phenotype.[131]

Another important variant is observed when a leukemia has more than one type of leukemic blast population in the bone marrow (i.e., mixed populations of blasts that are either lymphoid or myeloid). When these findings are observed in conjunction with consistent morphology, histochemistry and molecular changes (i.e., T-cell receptor or B-cell immunoglobulin gene rearrangements), then the diagnosis of a mixed lineage leukemia can be made.[132,133] The new WHO classification proposal is to refer to such cases as *bilineal leukemia*.[105,106,108] In some circumstances, the conversion from ALL to AML or vice versa may occur in mixed lineage leukemia after treatment. This may occur even though the chromosomal abnormality observed at diagnosis is preserved, possibly as a result of selection of one population as being resistant to initial therapy or to the true plasticity of such cases in terms of their capacity to differentiate along two different lineages.[134] The best treatment of mixed lineage leukemia has not been definitively determined, but often hybrid treatment approaches (i.e., a combination of treatments for AML and ALL) have been utilized.[134,135 and 136]

Although most cases of mixed lineage leukemia are characterized by lymphoid and myeloid markers, there are also cases of more than one pattern of differentiation within the myeloid lineage. For example, cases of erythromegakaryoblastic (M6/M7) leukemia can occur, demonstrating some leukemic blasts with the red cell precursor marker, glycophorin, and other blasts with megakaryocytic markers, such as CD61. Such cases can be explained by the fact that these two lineages are known to share a common precursor that in turn shares expression of certain genes, such as GATA transcription factors.[137,138 and 139] Patients with this type of leukemia are treated with therapies for AML.

The definitive identification of the type of leukemia is usually made by a combination of morphology, histochemical staining, and the pattern of surface antigen expression as determined by immunophenotyping. Cytogenetics has also now taken on a more important role in classifying AML as well as in prognostication.

### Cytogenetics

Another increasingly important component of AML classification is the use of cytogenetic and molecular tools to detect specific chromosomal rearrangements. This depends on the specificity of the presence of these chromosomal changes with unique subtypes of AML. High-resolution chromosomal banding in conjunction with FISH and polymerase chain reaction (PCR) approaches have significantly improved the ability to detect more subtle chromosomal alterations.[140,141 and 142] In most examples thus far examined, the chromosomal translocations or other abnormalities result in the alternation of specific genes that play critical roles in cell survival, differentiation, cell cycle regulation, chromosomal repair, and stability as well as transcription (see the sections Molecular Mechanisms of Leukemic Transformation and Subtypes of Acute Myelogenous Leukemia).[93,143,144] For example, the t(15;17)(q22;q21) translocation is diagnostic of APL. The t(8;21) translocation is more frequently observed in M2 AML, whereas inv(16) abnormalities are commonly found in a variant of myelomonocytic leukemia characterized by the presence of abnormal, basophilic granules (M4Eo). The contribution of cytogenetic and molecular changes to the classification of AML is continuing to grow. A summary of some of these changes, including altered genes and the subtype of AML most commonly associated with the genetic abnormality, is shown in Table 20-4.

**TABLE 20-4. CYTOGENETIC ABNORMALITIES IN CHILDHOOD ACUTE MYELOGENOUS LEUKEMIA (AML)[a]**

The combination of clinical presentation, morphology, histochemistry, immunophenotyping, and cytogenetics currently forms the basis for identifying the subtypes of AML. Although classification schema have had relatively limited relevance to determining therapy or prognosis, more recent subtype classification has begun to take on much greater importance in terms of directed therapy as well as predicting outcome. In addition, subclassification using genomic and RNA expression microarray technologies will clearly have a major impact on the ability to classify and determine appropriate molecularly directed therapies in the future.[145]

## Molecular Mechanisms of Leukemic Transformation

The molecular pathogenesis of AML remains incompletely understood. Most of the molecular insights into leukemic transformation have been derived from cytogenetic analysis. The cloning of chromosomal translocation breakpoints common in AML has demonstrated that the mechanism of AML transformation is distinct from the pathogenesis of other types of leukemia. For example, whereas ALL is most commonly characterized by overexpression of structurally normal transcription factors through juxtaposition of these genes into the genetic loci of highly expressed immunoglobulin or T-cell receptor genes, AML is most frequently associated with the generation of chimeric fusion genes that result from chromosomal translocation. Again, transcription factor encoding genes appear to be the most commonly targeted class of genes that are rearranged by such translocations in AML. For example, the t(8;21) results in fusion of the AML1 gene on chromosome 21 to the ETO gene on chromosome 8, yielding an AML1-ETO fusion protein that retains the DNA-binding properties of AML1 and the protein interaction properties of ETO. The AML1-ETO fusion protein is thought to block the normal function of the normal AML1 protein, part of the core binding factor complex whose activity is critical for normal hematopoietic development.

When considered at the level of cytogenetic analysis, AML appears to be a highly molecularly heterogeneous disease in that a multitude of distinct chromosomal abnormalities are seen in different patients with AML. Further characterization of the molecular consequences of such chromosomal translocations, however, reveals that in fact multiple different translocations actually target the same molecular pathway. For example, the t(8;21), t(3;21), t(16;16), t(16;21), and inv(16) translocations all result in dysregulation different of components of the core binding factor complex described above. In general, it appears that these translocations result in conversion of the normal core binding factor complex, which functions to activate the expression of target genes, into a transcriptional repressor, which functions to silence the activity of those target genes. This occurs through the recruitment of histone deacetylases (HDACs), which function as part of the transcriptional repression apparatus. Other translocations, such as the t(15;17), which results in the PML-RARA fusion seen in APL, also appear to cause leukemic transformation through aberrant recruitment of HDACs that cooperate in the silencing of genes normally activated by the normal RARA nuclear hormone receptor. This convergence of multiple gene rearrangements on a small number of common final pathways has significant therapeutic implications. Rather than having to develop a therapeutic intervention for each of the genetic abnormalities associated with AML, it may be possible to target only a few commonly dysregulated pathways. For example, the development of HDAC inhibitors has been considered as potential “transcription therapy” for AML. Whether such strategies will be effective and sufficiently specific remains to be determined.

Although many cases of AML appear to have only a single cytogenetic abnormality, it is becoming increasingly clear that multiple genetic mutations or “hits” are likely to be required for complete leukemic transformation. For example, in some patients with t(8;21)-positive AML, PCR evidence of the AML1-ETO fusion transcript persists even when patients are in complete remission (CR), suggesting that these patients may have returned to a premalignant state. Further support for this notion that the t(8;21) is not sufficient for leukemic transformation includes data coming from animal models that clearly demonstrate that the AML1-ETO fusion protein is not sufficient to cause leukemia in mice; additional mutations are required to reveal an overt leukemia phenotype.

## Subtypes of Acute Myelogenous Leukemia

The M0 subtype (minimally differentiated AML) comprises a small proportion (less than 3%) of pediatric AML. The leukemic cells are large and usually devoid of characteristic granules (type I blast). Although the blasts are negative for MPO staining with light microscopy, they may show positivity at the electron microscopic level. M0 AML is best defined based on the expression of myeloid-associated markers, such as CD33, CD13, and CD117 (c-Kit), in the absence of definitive evidence of lymphoid differentiation. Most cases are CD34 positive, whereas other markers, such as CD7 or deoxynucleotide transferase, may also be expressed.

The M1 subtype comprises approximately 20% of AML and is characterized by minimal evidence for differentiation. M1 is similar to M0 AML except for the presence of MPO as detected by immunohistochemistry or flow cytometry. More than 90% of the cells in the marrow are myeloblasts, and at least 3% of cells are MPO positive. The presence of Auer rods is variable. The type I blasts are similar to those in M0, and other blasts, usually a minority, may contain up to six azurophilic granules and a lower nucleus to cytoplasmic ratio (type II blasts). Immunophenotyping usually shows positive staining for CD13, CD15, CD33, and CD34.

The M2 subtype represents AML with differentiation. This subtype comprises nearly 30% of patients with AML. The blasts may vary considerably in size and shape, have prominent nucleoli, and usually a pale blue cytoplasm with azurophilic granules and often Auer rods. Greater than 10% of the leukemic blasts must show evidence of differentiation, with less than 20% of these showing monocytic features. The t(8;21) chromosomal translocation is more commonly observed in this subtype, with a frequency of between 10% and 15%.[143,144]

M3 subtype (also referred to as *APL*) represents approximately 5% to 10% of childhood AML and is characterized by extensive promyelocytic differentiation, often with bilobed nuclei and Auer rods. A variant of M3 AML (M3v) is characterized by being micro- or hypogranular. However, these cells are strongly MPO positive. Thus, the M3v subtype of AML should be considered when the bone marrow contains many cells with a hypogranular appearance but that are strongly positive for the peroxidase reaction. This variant may constitute up to 25% of all cases of APL. Nearly all cases of M3 AML, including the microgranular variant, are characterized by the presence of a t(15;17) chromosomal translocation, which involves the fusion of the PML gene on chromosome 15 to the retinoic acid receptor alpha (RARA) on chromosome 17.[143,144] Importantly, this translocation is absent from other subtypes of AML. Thus, the presence of a t(15;17) chromosomal translocation, detected by routine cytogenetics, FISH, or RT-PCR, essentially makes the diagnosis of APL. However, an important cytogenetic variant, t(11;17), has been described that fuses the PLZF gene on chromosome 11 to the RARA gene. These different translocations have particular prognostic significance in terms of response to all- *trans*-retinoic acid (ATRA), with the t(15;17)-positive leukemia showing excellent responses and the t(11;17)-positive leukemia being mostly resistant. [143,144,146,147,148] and [149]

The M4 subtype of AML, called *acute myelomonocytic leukemia*, accounts for approximately 25% to 30% of AML, with an increased frequency in children younger than 2 years of age. M4 AML is characterized by the bone marrow having at least 20% of myeloblasts, whereas monoblasts comprise 20% to 80% of the nonerythroid cells, most commonly promonocytes. There is often a peripheral blood monocytosis (greater than $5 \times 10^9$ per L monocytes) as well as greater than threefold increased serum and/or urine lysozyme levels. In the case in which less than 20% of the bone marrow cells are monocytic, the diagnosis of M4 AML can be made in part based on the peripheral monocytosis. The myeloid blasts of M4 AML are usually MPO and SBB positive and express characteristic surface antigens such as CD13, CD15, and CD33. The monocytic blasts are NSE positive and, in addition to myeloid surface markers, also usually express CD14, CD4, and HLA-DR. A variant of M4 AML, called *M4Eo*, is characterized by the presence of greater than 5% abnormal eosinophilic precursors, which have large, basophilic granules in their cytoplasm. They are strongly chloroacetate esterase positive. This variant is strongly associated with alterations of chromosome 16, most commonly inv(16)(16q22). This chromosomal alteration usually involves the creation of fusion transcript involving the MYH11 and CBFB core binding transcription factor. Some cases of 16q22 abnormalities do make for strict FAB criteria for M4Eo subtype, and have previously been classified as M2 or M5. The new WHO classification should eliminate some of this potential confusion, as 16q22 leukemia is to be considered a unique entity. [104,105,106,107] and [108]

M5 AML (monocytic) can be distinguished from other subtypes, specifically the M4 subtype, by the presence of greater than 80% of the nonerythroid bone marrow cells being of the monocytic lineage. M5 AML represents approximately 15% of AML in children older than 2 years but may account for approximately 50% of AML in children younger than the age of 2 years. This subtype is divided into two subcategories, M5a, in which more than 80% of the bone marrow cells are immature monoblasts, and M5b, in which a mixture of monoblasts and promonocytic and monocytic cells comprise the 80% of cell types. The M5a blasts are characteristically large, contain prominent, multiple nucleoli, a basophilic cytoplasm, and usually lack Auer rods. The M5b subtype shows increased evidence of differentiation with more extensive granulation and sometimes the presence of Auer rods. The blasts are NSE positive and express myeloid surface markers, such as CD33, along with antigens characteristic of the monocytic lineage such as CD14. The t(9;11) chromosomal abnormality is commonly found associated with M5 AML and involves the translocation of the interferon beta1 gene into the MLL gene locus at 11q23-24. [143] In addition, the t(8;16) translocation has been observed in both M4 and M5 AML. This translocation involves the fusion of the MOZ and CBP genes. [143]

The M6 subtype of AML, called *erythroleukemia*, is observed in less than 5% of pediatric cases, but is more commonly observed in adults with secondary AML. [150] M6 AML can be diagnosed when at least 50% of the bone marrow cells are erythroblasts and the nonerythroid bone marrow elements contain 30% blasts. DiGuglielmo's syndrome is a relatively infrequently found subtype of M6 AML that is characterized by greater than 70% of the bone marrow cells displaying highly dysplastic and megaloblastic erythroblasts, often with multiple nuclei. Erythroblasts are strongly PAS and carbonic anhydrase positive. Myeloblasts are MPO and SBB positive. Immunophenotyping is positive for glycophorin.

M7 AML, referred to as *megakaryocytic*, represents less than 3% of adult AML and approximately 5% to 10% of AML in children, with the exception of children with DS younger than 2 years of age when M7 is the most common form of myeloid leukemia.[59,61] Morphologically, M7 AML can be confused with FAB class L2 ALL. The blasts vary in size with nuclei containing finely dispersed chromatin and multiple nucleoli. The cytoplasm is usually abundant and characterized by extensions of blebs similar to that observed with the budding of platelets. Often, the blasts may have clusters of platelets associated with them. The bone marrow may also contain

significant amounts of fibrosis and can result in difficult aspiration attempts, often resulting in “dry” taps. The blasts are negative for MPO and SBB reactivity, but may show localized positive reactions for alpha-naphyl butyrate esterase. Platelet peroxidase can be demonstrated at the electron microscopic level. A key feature to establish this diagnosis is immunophenotyping, which shows the blasts to be positive for CD13 and CD33 along with the megakaryocytic markers, such as glycoprotein IIb/IIIa (CD41) and glycoprotein IIIa (CD61). Factor VIII can also be detected by immunocytochemistry. The t(1;22) translocation has been described to be found in very young patients with M7 AML.[151,152 and 153]

The classification systems being currently used employ features of morphology, histochemical reactions, surface immunophenotyping, cytogenetic abnormalities detected using a variety of methods ranging from classical cytogenetics to molecular methods, such as FISH and PCR approaches to detect chromosomal abnormalities. It is important to realize that with the exception of the M3, M4Eo, and possibly M6/M7 subtypes, the classification schema has limited utility in terms of directing therapies and outcome. An important goal of future studies will be to develop a more detailed and predictive classification schema for malignant myeloid diseases that will help target more effective therapies.

## CLINICAL PRESENTATION OF ACUTE MYELOID LEUKEMIAS

The range of presenting signs and symptoms of children with AML is exceptionally large. Patients may present with minimal symptoms or life-threatening complications due to depletion of normal bone marrow elements and organ dysfunction based on leukemic cell infiltration. Of further importance is that certain subtypes of AML have characteristic presenting signs and symptoms.

### Presenting Signs and Symptoms

Persistent fevers may occur in approximately a third of patients, and are probably secondary to pyrogens released by leukemic cells and/or by macrophages and lymphocytes reacting to the leukemic blasts. Patients may also present with fever secondary to bacterial infections of the sinuses, gingiva, teeth, lung, perirectal area, skin, and septic shock as a result of neutropenia. Although the white blood cell count (WBC) is usually elevated in patients with AML, the number of functional, mature neutrophils is often significantly decreased to less than 1,000 cells per mL.

Pallor can result from decreased hemoglobin (usually a normocytic, normochromic anemia) due to leukemic blast infiltration into the bone marrow, entrapment from enlarged liver and spleen or from blood loss secondary to bleeding as a result of thrombocytopenia, and/or disseminated intravascular coagulopathy (DIC). Additional consequences of severe anemia include fatigue, headache, tinnitus, dyspnea, and congestive heart failure. Bone pain, which occurs in close to 20% of patients, is a result of bone marrow replacement by leukemic blasts; patients may present with limp, long bone or rib pain, as well as back pain.

Some degree of hepatosplenomegaly occurs in approximately one-half of patients and is due to organ infiltration, with the exception of very young children with DS and M7 AML, who may present with hepatomegaly and liver failure due to fibrosis in addition to leukemic involvement. [56] Extramedullary involvement with AML may also result in lymphadenopathy (between 10% and 20% of patients); leukemia cutis (less than 10% of patients), characterized by palpable, nontender nodules or plaques that are colorless to bluish/purplish in color; and gingival hypertrophy (in 10% to 15% of patients); all of these findings are more common in infants and patients with M4 or M5 AML.[154,155] The growth of tumors composed of AML blasts, called *granulocytic sarcomas* or *chloromas*, the latter name because of their bluish-green appearance due to the MPO content, may also occur in a variety of anatomical sites. Chloromas are most commonly found in the orbit or periorbital areas where they can cause ptosis. They may also involve the spinal cord and cause cauda equina syndrome or paraparesis from epidural involvement. [156,157,158,159,160 and 161] Isolated chloromas or granulocytic sarcomas can precede the bone marrow involvement by just a few weeks to up to 1 or 2 years.[162] Involvement of the central nervous system (CNS), which occurs in approximately 2% of patients, can be in the form of leukemic cells in the cerebrospinal fluid or chloromas from which patients can present with headache, nausea and vomiting, photophobia, papilledema, and cranial nerve palsies. [159,163,164,165 and 166] Seizures are not common. CNS involvement is more common in infants and patients with M4 and M5 AML as well as those presenting with a very high WBC. Testicular involvement rarely occurs with AML. [167,168 and 169]

### Bleeding

Petechiae and purpura are a result of thrombocytopenia and sometimes coagulopathy. Overt bleeding may involve the large or small bowels, oral mucosa, or CNS; nose bleeding and menorrhagia may occur. These hemorrhagic complications may result from thrombocytopenia as well as from DIC due to infection or from the release of proteins with anticoagulant activities (i.e., thromboplastin activity) from cytoplasmic granules of leukemic blasts. DIC is most frequently observed in M3 AML (APL) because of the high level of the thromboplastin activity containing granules in promyelocytes. Importantly, the induction of therapy may worsen DIC due to the breakdown of leukemic blasts.[170,171 and 172] The use of low-dose heparin has been proposed by some investigators during initial treatment. [173,174 and 175] The use of ATRA has provided an alternative means to induce remission by the further differentiation of the promyelocytes followed by apoptosis without the acute lysis of leukemic blasts. However, coagulopathy and bleeding complications may still be problematic (see Hematologic Complications).[176,177 and 178] M5 AML is also frequently associated with DIC secondary to the release of proteins with anticoagulant activities.

### Hyperleukostasis

When the peripheral WBC reaches very high levels, usually greater than 200,000 cells per mL, leukemic blasts can start to clump intravascularly, resulting in the condition of leukostasis. This medical emergency occurs as a result of sludging of clumped leukemic blasts in the small vessels and may lead to tissue hypoxia, infarction, and hemorrhage. Lung involvement can result in significant tachypnea and eventual respiratory failure; chest x-rays often show parenchymal infiltrates, engorged vessels, and pulmonary edema. CNS involvement can result in confusion, headache, somnolence, coma, and stroke. Most children will not develop signs or symptoms of leukostasis with WBC under 200,000 cells per mL, but the risk of developing severe complications, such as CNS hemorrhage and pulmonary failure, increase substantially with WBC over 300,000 cells per mL. [172,179,180,181 and 182] Some studies have indicated that children with WBC of greater than 100,000 cells per mL with M5 AML and extramedullary organ involvement may be at greater risk.[172] This may be due to the large size and adherence properties of monoblasts. [172]

### Tumor Lysis Syndrome

Tumor lysis syndrome results when the intracellular contents released from dying leukemic blasts exceeds the ability of the body to adequately metabolize and excrete them. This is most frequently associated with AML presenting with a very high WBC. Hyperuricemia, a result of the metabolism of excess amounts of released nucleic acids, can result in renal failure, which then can further worsen the patient's ability to excrete other metabolites. Increased blood urea nitrogen can adversely affect platelet function and lead to a worsening of any coagulopathy. Hyperphosphatemia and secondary hypocalcemia may lead to further renal failure and the danger of seizures. Hyperkalemia may lead to alterations in the electrocardiogram with increase amplitudes of T waves, arrhythmia, and cardiac arrest.

### Laboratory Findings

A complete blood cell count will show a hemoglobin of less than 9 g per dL in more than one-half of patients, with a range between approximately 2.5 and 14.0 g per dL (median of 7 g per dL).[183] The anemia is usually normocytic and normochromic, but can show teardrop-shaped RBCs and circulating nucleated RBCs, both signs of marrow infiltration. Platelet counts of less than 100,000 platelets per mL occur in nearly 75% of patients. [183] The median leukocyte count is 24 × $10^9$ cells per mL, with up to approximately 20% of patients presenting with a WBC greater than 100,000 cells per mL. [172,184] Sweet's syndrome can be encountered in patients presenting with AML.[185] As noted, hyperuricemia, hyperkalemia, hyperphosphatemia, and hypocalcemia may be mild to severe depending on the leukemic burden and leukemic cell turnover rate. Patients with M3 AML (APL) and M5 AML (monocytic) are more likely to show signs of DIC with associated laboratory abnormalities such as prolonged prothrombin time, partial thromboplastin time, and decreased fibrinogen. Patients with M4 and M5 AML may also show increased serum and urine levels of lysozyme, an enzyme stored in the cytoplasm of monocytic blasts. Of interest is that this enzyme may lead to renal tubular dysfunction. [186]

### Differential Diagnosis

The differential diagnosis of AML includes both nonmalignant and other malignant conditions. The former include such disorders as juvenile rheumatoid arthritis, infectious mononucleosis, aplastic anemia, congenital and acquired neutropenia, autoimmune cytopenias, megaloblastic anemia, leukemoid reactions secondary to severe infections or hemolysis, conditions such as infection or marrow infiltrative disorders leading to leukoerythroblastic peripheral blood smears, and transient myeloproliferative syndrome associated in infancy with trisomy 21. Depending on the age and presentation, malignant disorders may include metastatic

neuroblastoma, rhabdomyosarcoma, retinoblastoma, non-Hodgkin's lymphoma, or ALL, as well as MDS and subacute or chronic myeloid leukemias, including juvenile myelomonocytic leukemia, chronic myelomonocytic leukemia, and CML.

A definitive diagnosis of leukemia and type should be made by examination of cells obtained by bone marrow aspirate or biopsy. Usually, a bone marrow aspirate is adequate and a biopsy unnecessary. However, a biopsy is useful in the situation in which leukemia is highly suspected but aspirate attempts do not yield sufficient material for examination, so-called “dry” taps. The bone marrow in AML is usually hypercellular and should contain greater than 25% leukemic blasts. Histochemistry immunophenotyping along with cytogenetic analysis is usually able to definitively distinguish AML from the previously mentioned conditions as well as determine the subtype. If there is still doubt whether a patient has leukemia or not, then most patients can usually be safely watched for approximately another week, at which time a repeat bone marrow examination can be done. In addition to the bone marrow aspirate, the work-up of patients suspected of having AML should include a complete blood cell count and differential; blood type and cross if indicated; coagulation studies; blood chemistries, including electrolytes, uric acid and liver and renal function tests; and a lumbar puncture.

## TREATMENT: REASONS FOR SUCCESS AND FAILURE

The goal of therapy for patients with AML is to eradicate their leukemia while allowing them to lead normal lives. The foundation stones of successful treatment for patients with AML currently include combination chemotherapy, as performed in carefully controlled clinical trials, along with aggressive supportive care. Before 1970, nearly all patients with AML died of their disease. Initial attempts to treat patients with AML were modeled on the first successful approaches used in ALL (i.e., induction therapy followed by prolonged maintenance treatment and cranial radiation). Subsequent improvements arose out of the more intensive dosing of multiple, chemotherapeutic agents with non–cross-resistant drug profiles, leading to transient but severe hypocellular bone marrow. [187,188] Studies done in the 1970s and 1980s that were based on these principles led to a shorter duration of therapy without the need for cranial radiation and resulted in improved remission rates and long-term outcomes, with 5-year survivals reaching the 25% to 35% range (Fig. 20-3).[189]

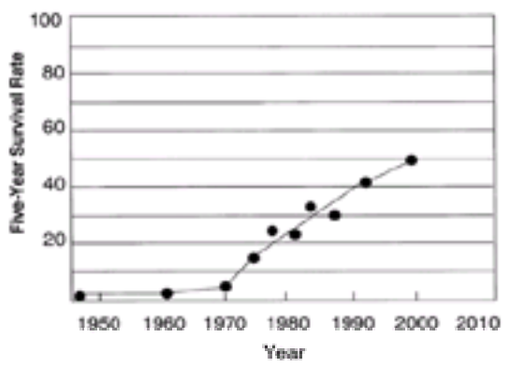


**FIGURE 20-3.** Five-year survival in pediatric acute myelogenous leukemia over the past 50 years. [Adapted from Kersey JH. Fifty years of studies of the biology and therapy of childhood leukemia. Blood, 90:4243;1998; and SEER data (Smith MA, Gloeckler-Ries LA, Gurney JG, Ross JA. Leukemia. In: Ries LAG, Smith MA, Gurney JG, et al., eds. Cancer incidence and survival among children and adolescents: United States SEER Program 1975–1995. Bethesda, MD: National Cancer Institute, SEER Program NIH Pub., 1999:17–34.).]

Building on these concepts, subsequent studies have continued to take advantage of dose intensification using chemotherapy along with autologous or allogeneic bone marrow transplantation. Overall, long-term survival has now been achieved for 45% to 50% of patients with AML. [189] Several important lessons have been learned from such studies, including the importance of dose intensification during the early phases of therapy as well as the role of allogeneic bone marrow transplantation.[190,191 and 192] In addition, the treatment of patients with different subtypes of AML on standardized clinical trials has begun to identify subsets of patients for whom alternative approaches are more efficacious.[193,194 and 195] However, these important advances have come at a significant cost in terms of short- and long-term morbidity and mortality, which have concomitantly increased with the escalation of dose intensification. An increasingly important concern linked to toxicity is the pharmacogenetic basis for drug sensitivity due to the expression of polymorphisms of genes involved in drug metabolism. [196,197] For example, prescreening of patients for such polymorphisms may help to identify patients at particularly high risk for specific toxicities and aid in risk-adapting therapeutic choices. Resource utilization has also significantly increased. Furthermore, despite both the success and the associated treatment related problems, the most common reason for treatment failure in patients with AML remains drug-resistant leukemia.[198,199 and 200] There remains an enormous need for innovative, less toxic, and more effective approaches to treat patients with AML.

Current therapy for AML involves the stabilization of the patient at the time of diagnosis followed by remission-induction therapy and postremission treatment that includes consolidation, intensification, and CNS prophylaxis. In some studies, maintenance therapy is used. This section discusses each of these issues in terms of both progress and unfulfilled challenges.

### Immediate Therapy

The diagnosis of AML often constitutes an urgent situation, and patients should undergo careful evaluation for the hematologic, infectious, and metabolic consequences of their leukemia.

#### *Hematologic Complications*

Thrombocytopenia in a patient with AML takes on special significance in terms of the potential for catastrophic bleeding due to the increased risk of bleeding, even with relatively high platelet counts, secondary to DIC and infection. In such circumstances, platelet counts should be maintained at higher levels than in patients without such risk factors. The goal should be to maintain hemostasis and not necessarily achieve a specific platelet count. Patients with DIC should receive replacement clotting factors through the judicious use of fresh frozen plasma, platelet transfusions, and, on occasion, clotting factor concentrates. The efficacy of using low-dose heparin as prophylaxis and management of DIC secondary to APL remains unproven.[173,174 and 175,178] The use of ATRA for induction in such patients may improve the exacerbation of the coagulopathy associated with induction chemotherapy. However, hemorrhagic complications still occur. Patients with APL and high WBCs are particularly at risk for significant hemorrhage. [176,177] In such circumstances, the immediate goal, in addition to managing the coagulopathy, is to reduce the leukemic burden.

Anemia in patients with AML may be severe and due to the replacement or suppression, or both, of normal red cell production as well as bleeding due to thrombocytopenia and/or DIC. Like the platelet count, it is important to maintain adequate hemoglobin by anticipating blood loss in patients with active bleeding and DIC (see Chapter 40).

#### *Infectious Complications*

Although the WBC count may be high in patients with AML, the absolute neutrophil count is often less than 1,000 cells per mL. When the neutrophil count drops to below 200 cells per mL, the incidence of fever and bacteremia increases dramatically. The low neutrophil count in conjunction with fever, present in at least one-third of patients, is a medical emergency. Patients presenting with fever and neutropenia should have blood and urine cultures sent and be promptly started on therapy with broad-spectrum antibiotic coverage.[201,202,203,204,205 and 206] The risk of developing fungal infection during induction therapy for AML has increased along with the intensification of therapy. Although some studies have suggested the use of antifungal agents as prophylaxis, this is an area of investigation requiring more study, especially in terms of *Aspergillosis*.[207,208,209,210,211 and 212] Cytokines aimed at accelerating the rate of neutrophil recovery have proven to be effective, but have not been unequivocally shown to improve overall outcome and reduce infections in randomized studies. [213,214,215,216,217,218,219,220,221 and 222]

### *Tumor Lysis Syndrome*

The presenting signs and symptoms of tumor lysis syndrome may include renal failure, increased serum uric acid, potassium, and phosphate with secondary hypocalcemia.[223,224] These patients require immediate intervention with intravenous hydration to maintain urine flow, alkalinization with intravenous sodium bicarbonate, and allopurinol or other agents to increase the solubility and renal excretion of insoluble urate. [224,225 and 226] Frequent monitoring of serum electrolytes, in addition to potassium, calcium, phosphorus, creatinine, and urine output, is critical. Although the renal failure associated with tumor lysis syndrome can usually be avoided with anticipatory measures of hydration and administration of allopurinol, occasionally such patients will require dialysis until renal function is reestablished.

### *Hyperleukostasis*

Patients presenting with WBC counts greater than 200,000 cells per mL are at increased risk of developing respiratory insufficiency and CNS complications such as headache, confusion, somnolence, coma, and hemorrhage. Under such circumstances, it is imperative that rapid cytoreduction take place. Leukapheresis or exchange transfusions are able to rapidly lower the WBC, although the effect is usually transient. [172,179,180,181 and 182] Hydration also causes a decrease in WBC due to dilution. The use of cytotoxic agents, however, is critical for effectively sustaining a reduced WBC. Agents such as hydroxyurea can be immediately given while preparing for leukapheresis, and, though the effect of this agent may not be manifest for several hours, it may both lower and prevent a rebound increase in the leukemic blast count after cytapheresis. More definitive cytoreductive therapy (see next section) should be introduced as soon as possible. The use of radiation therapy is not indicated for hyperleukostasis, but may be helpful in the situations in which chloromatous accumulations of leukemic blasts threaten organ function such as with involvement of cranial nerves, eyes, spinal column, or trachea.

## Induction Therapy and Complete Remission

Induction therapy should begin as soon as possible after a definitive diagnosis is made. Although initial intervention is important in metabolically and hematologically stabilizing the patient, the initial cytoreductive therapy is key to inducing a remission and obtaining a return to normal hematopoiesis. Remission is currently defined as the presence of less than 5% blasts in a normocellular marrow with trilineage recovery of peripheral blood counts (granulocyte count of greater than $1 \times 10^9$ cells per L and platelet count greater than $100 \times 10^9$ per L) as well as no evidence of leukemia in other sites. It is important to determine differences in remission criteria when comparing different studies, as these criteria are not used to report results in all studies. [119,227] Some studies have demonstrated that the quality of remission after induction therapy is likely to have a profound impact on outcome regardless of the type of postremission treatment. Although “quality” has not yet been rigorously defined, it correlates closely with the degree of dose intensification and probably the degree of MRD (see under Response to Therapy and Minimal Residual Disease).

The induction of a remission currently requires the use of myelosuppressive cytotoxic agents that result in transient periods of profound marrow hypoplasia. An exception to this is the induction of remission in patients with APL, in whom the use of agents, such as ATRA, allow for the terminal differentiation and apoptosis of leukemic blasts while effectively sparing normal myeloid progenitors (see the section Acute Promyelocytic Leukemia). The periods of hypoplasia and secondary pancytopenia usually last from 20 to 30 days, during which time patients are at high risk for infection and bleeding. Furthermore, the damage to normal tissues, particularly the gastrointestinal tract, creates a significant source for entry of bacterial organisms into the blood stream. Last, intensive chemotherapeutic treatments inhibit lymphocyte function, resulting in various degrees of susceptibility to opportunistic infections. Thus, anticipatory supportive care measures are critical for patients to survive the consequences of induction therapy.

After the induction of remission in patients with ALL using the antimetabolite aminopterin in the late 1940s, the National Cancer Institute sponsored the screening of many cytotoxic agents against leukemia cell lines. In the 1960s, cytarabine (AraC) and anthracyclines were introduced into the treatment of patients with AML, initially as single agents and then in combination. With the introduction of the “7 and 3” regimen consisting of a 7-day intravenous infusion of 100 mg per $m^2$ AraC along with 3 days of bolus daunomycin at 45 mg per $m^2$ per dose, approximately 60% to 70% of patients with newly diagnosed AML may achieve remission. [190,191,228,229,230 and 231]

The addition of other agents to the “7 and 3” regimen or the use of multiple agents sequentially over the course of a week demonstrated remission induction rates from 70% to 85%.[59,188,191,228,232,233,234 and 235] Some studies have demonstrated that the addition of etoposide to “7 and 3” increases the duration of remission. [191,229,236,237 and 238] On the other hand, another study by the Cancer and Leukemia Group B did not observe an increased remission rate or duration with the addition of 6-thioguanine (6-TG) to “7 and 3.”[239] In a randomized trial from the United Kingdom's Medical Research Council (MRC AML10), which compared daunomycin and AraC with either 6-TG or etoposide, no significant difference was observed. [240]

The use of alternative anthracyclines, such as idarubicin (IDA), has suggested remission induction can be achieved sooner and in a greater number of patients than with “7 and 3” using daunomycin.[191,241,242,243,244,245,246 and 247] The MRC/ICRF performed a meta-analysis of results from randomized trials comparing IDA or mitoxantrone versus daunomycin and found that there was a statistically significant improvement in remission rate with IDA and a trend that mitoxantrone was better than daunomycin.[248] The overall disease-free survival, however, was not different among the different groups, although a detailed analysis by age was not done in spite of the fact that the improvement remission induction observed with IDA suggested that there was more of an advantage in younger patients. [248] Other trials have not achieved higher remission rates by introducing IDA.[191,247,249] Part of these discrepancies may involve the issue of dose equivalency, which remains an important variable in studies of this type.

Increasing the dose of AraC during induction has not been shown to consistently result in a greater remission induction rate, but has contributed to increased toxicity, particularly in older patients.[250,251] For example, randomized studies in adults with AML have shown that no significant differences were observed with AraC at 100 to 200 mg per $m^2$.[252,253] Further increases of AraC dose, by even 20 to 30 times (i.e., 1,000 to 3,000 mg per $m^2$ every 12 hours for eight to 12 doses) have also not resulted in significantly improved remission rates. [250,254,255]

Thus, the percentage of patients with AML achieving remission remains between 70% and 85%, with approximately one-half of the failures being due to resistant leukemia and the others to toxicity. Such numbers can, however, be misleading in that remission rates may vary significantly depending on whether patients have favorable or unfavorable characteristics. For example, remission rates may be significantly higher (greater than 80%) in favorable risk patients [e.g., (inv)16 AML] whereas subsets of patients with poor risk factors (e.g., older patients, antecedent MDS, and adverse cytogenetics such as monosomy 7) usually have remission rates below 50% (see the section Prognostic Factors).[251,256] Thus, although “7 and 3”-based regimens have been reasonably successful, they remain significantly limited.

In an attempt to improve remission rates, several studies have more recently tried to build on the observation that AML has been responsive to dose intensification as well as the concept that kinetically based approaches might result in increased leukemic cell recruitment and killing. [191,257,258,259 and 260] Both of these concepts have led to different strategies for dose intensification. In one case, chemotherapy is given on a daily schedule over a contiguous series of days. [239,240,261,262] The other approach delivers chemotherapy over a similar period, but separates the treatment by a gap to provide time for the recruitment of leukemic blasts into a potentially more drug-sensitive, proliferative state [e.g., Children's Cancer Group (CCG) study 2891]. [60,192,257,258,263,264]

The Cancer and Leukemia Group B demonstrated that a “7 and 3” induction was better than a “5 and 2” induction regimen. [261] The MRC AML 9 trial, comparing a “10 and 3 plus 6-TG” induction with a “5 and 1 plus 6-TG,” showed that the longer infusion (i.e., 10 days of AraC) had a higher CR rate. [261] Based in part on these results, the MRC AML 10 trial used AraC for 10 days during induction and compared the addition of etoposide or 6-TG. [240] Whether one used etoposide or 6-TG, this trial resulted in a remission induction rate of approximately 85% after one or two courses of therapy and as high as 92% with four cycles of treatment ( Fig. 20-4).[240] The Leucemia Acuta Mieloide (LAM) 89/91 study used AraC at 1,000 mg per $m^2$ for 5 days and replaced the standard 3 days of daunomycin with 5 days of mitoxantrone (Fig. 20-4). This study showed a remission induction rate of 87% and represented a significant dose increase of both AraC and anthracycline. [265] The Berlin-Frankfurt-Munster (BFM) trials (BFM 83 and 87) also increased exposure to AraC during the first 14 days of treatment and demonstrated a similar remission induction rate to other studies.[266,267,268,269 and 270] Such studies have thus dose intensified, in part, by extending the period of induction therapy as a period of consecutive daily treatments. This has resulted in a trend toward slightly higher remission induction rates.

**FIGURE 20-4.** Comparative schema from pediatric randomized trials. A, cytarabine; ADE, AraC, daunorubicin, and etoposide; AIEOP/LAM, Associazione Italiana di Ematologia ed Oncologia Pediatrica; AraC, cytarabine; Aza, 5-azacytidine; Auto HSCT, autologous hematopoietic stem cell transplantation; BFM, Berlin-Frankfurt-Munster; CCG, Children's Cancer Group; CLASP, cytarabine, L-asparaginase; CPM, cyclophosphamide; D, daunorubicin; DAT, daunorubicin, AraC, and thioguanine; Dauno, daunomycin; DCTER, dexamethasone, cytarabine, thioguanine, etoposide, and rubidomycin; DOX, doxorubicin; E, etoposide (VP-16); Famp, fludarabine; HAD, high-dose AraC and daunorubicin; HAM, high-dose AraC and mitoxantrone; HDAraC, high-dose AraC; HiDAT, DAT with AraC given at 1 g per $m^2$ q12h × 14 doses instead of 100 mg per $m^2$ per day for 7 days; I, idarubicin; IDA, idarubicin; IL2, interleukin-2; L-Asp, L-asparaginase; LAM, Leucemia Acuta Mieloide; M, mitoxantrone; MACE, m-amsa (amsacrine), cytarabine, and etoposide; MEC, mitoxantrone, etoposide, and cyclosporin A; MiDac, mitoxantrone and cytarabine; Mito, mitoxantrone; MRC AML, United Kingdom's Medical Research Council acute myelogenous leukemia study; MRD HSCT, matched related-donor hematopoietic stem cell transplantation; MSD, matched sibling donor; POG, Pediatric Oncology Group; Pred, prednisone; Rx, treatment; St'd, standard; TAD, thioguanine, AraC, and daunorubicin; 6-TG, 6-thioguanine; VCR, vincristine; XRT, radiation treatment. [BFM-87 [268]; BFM-93 (Creutzig U, Ritter J, Zimmerman M, et al. for The Acute Myeloid Leukemia–Berlin-Frankfurt-Münster Study Group. Improved treatment results in high-risk pediatric acute myeloid leukemia patients after intensification with high-dose cytarabine and mitoxantrone: results of Study Acute Myeloid Leukemia-Berlin-Frankfurt-Münster 93. ICO 2001;19:2705–2713.) MRC AML 10 [322]; LAM 89/91[265]; AIEOP/LAM 87[323]; POG 8821[324]; POG 9421 (from POG Operations Office); CCG 2891[263,721]; CCG 2961 (from CCG Operations Office).]

An alternative strategy was based on the concept that leukemic blasts could be effectively recruited into S phase of the cell cycle by following the initial course of chemotherapy by a 6- to 8-day gap before reinstitution of therapy. This type of timed, sequential, or intensively timed therapy originated from work on leukemic cell cycle kinetic studies.[257] For example, the CCG-2891 trial tested a “standard” versus “intensive” timing induction using the five-drug dexamethasone, AraC, thioguanine, etoposide, and rubidomycin (DCTER) regimen.[60,264] Standard timing delivered the second course of chemotherapy after bone marrow recovery, usually approximately 30 days, unless there was residual leukemia on day 14, in which case, therapy was given at that time. Intensively timed induction therapy gave the second course of DCTER on days 10 to 13 regardless of bone marrow status (Fig. 20-4). The CR rate for the standard and intensive timing arm was 74% and 78%, respectively, a nonstatistically significant difference. [60,264] However, induction mortality was great on the intensively timed induction although there was less refractory leukemia. The addition of G-CSF, as a nonrandomized question part way through this study, showed that there were fewer infectious complications, resulting in a remission rate of 82%. Although these results showed that intensively timed induction therapy did not result in a significantly improved remission rate, follow-up information has demonstrated that there is a significantly improved overall outcome for patients who received the intensive versus standard timing therapy, regardless of the type of postremission treatment (see Postremission Therapy).[60,264]

The results from such studies strongly suggest that the type of therapy received during induction has an important impact on overall outcome. In addition, these results lead to the hypothesis that the quality of the remission obtained using intensively timed therapy was superior to that obtained with standard timing. In biologic terms, quality is believed to be a remission with less MRD. This is currently being tested in prospective trials (see Postremission Therapy).

Although many studies have shown that at least a subset of AML is more responsive to the intensity of therapy during induction, the issue of timing versus dose intensity has not been rigorously answered. For example, the MRC 10, BFM, and LAM studies, which give more therapy by extending the days of induction treatment, have achieved similar results to intensively timed therapy as demonstrated by the CCG-2891 trial. A definitive test of timing versus dose intensification should involve a comparison of the same amount of therapy delivered over the same time period with one group of patients receiving that therapy each day and the other group receiving part of the treatment followed by a gap (to account for kinetic recruitment) and then the rest of therapy ( Fig. 20-5). Future trials may help elucidate the answer to this question.

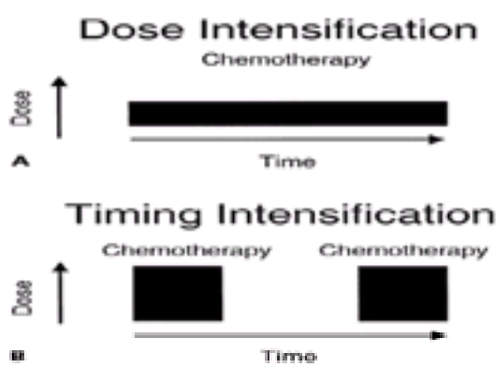


**FIGURE 20-5.** Schema of “dose versus timing” of chemotherapy. **A:** Chemotherapy extended over a contiguous number of days. As more cells cycle into different sensitive phases of the cell cycle, there would be an expected increased killing of leukemic blasts. **B:** A test of timing of the same total dose of chemotherapy as depicted in **A**, but instead given as two periods of chemotherapy, separated by a gap. This latter approach assumes that the first course of chemotherapy will both kill and partially synchronize leukemic blasts so that they will be in S phase and, possibly, in a more chemosensitive state when the second course of therapy is delivered.

Another critical component for success during remission induction has been the development of improved supportive care measures. [271,272] and [273] Although the role of broad-spectrum antibiotic coverage in patients who are febrile and neutropenic has been well established, the role of antibacterial prophylaxis and antifungal prophylaxis is not entirely clear. In addition, the role of hematopoietic growth factors has been extensively tested in patients with cancer after chemotherapy-induced neutropenia.[222,274,275] and [276] In patients with AML, there have been several studies examining the potential role of cytokines, such as G-CSF or granulocyte-macrophage colony-stimulating factor (GM-CSF), to reduce the period of neutropenia and serious infections. [274,275] These studies have demonstrated that the use of hematopoietic growth factors after induction and possibly consolidation therapy reduces the period of neutropenia, although the affect on the incidence of infections and hospitalization has been variable. [274,275,277] Some, but clearly not all, prospective randomized trials in elderly patients have demonstrated significantly improved survival with the use of GM-CSF due to a decrease in treatment-related deaths. [221,278] Similar studies using G-CSF have not shown significant clinical advantages, although minimal reduction in periods of neutropenia have been observed. [213,214,216,217,218,219,220,221] and [222,274,275] Of note, most studies have not shown a consistent effect on the remission induction rate, the duration of responses, or the incidence of relapse.

Although real progress has been made in improving remission induction rates, significant challenges remain. The combination of anthracyclines and AraC was introduced as a relatively effective combination in the 1970s and remains the mainstay of induction therapy. Dose intensification of induction therapy has achieved better quality remissions but at a significant cost in terms of morbidity and mortality. [279] Furthermore, increasing dose intensification with cytotoxic agents that are currently being used is likely to be too toxic. Advances in supportive care, particularly antifungal agents, are needed for incorporation into initial therapy. Future studies will need to take advantage of more specific antileukemic agents that do not contribute to normal tissue toxicity.

## Postremission Therapy

Without some type of postremission therapy, nearly all patients with AML relapse. This was clearly demonstrated by an Eastern Cooperative Oncology Group study.[280,281 and 282] The degree of intensity, scheduling, and duration have been examined in a number of important trials. [282,283] Early studies from Saint Jude Children's Research Hospital (SJCRH AML-76), the CCG (CCG-241), and the German cooperative groups demonstrated that postremission maintenance therapy resulted in a 20% to 25% 2-year relapse-free survival (RFS). [284,285,286 and 287] Essentially all patients who did not receive postremission therapy died from leukemia. Studies from the Dana-Farber Cancer Institute (DFCI), including the initial VAPA trial, attempted to intensify postremission therapy by delivering 14 months of sequential combination chemotherapy, resulting in an EFS of 38%.[288,289] The BFM-78 trial used 2 years of maintenance therapy after an induction and postinduction consolidation period and showed an EFS of 35%, although the role played by the maintenance phase remains unclear. [287]

Similar results were obtained by the Pediatric Oncology Group (POG-8101), which randomized patients to two different maintenance treatments lasting 2 years. [252] The 2-year disease-free survival (DFS) from that study did not differ from previous studies; no difference was observed between the two maintenance arms, which differed primarily in that one group received daunomycin/AraC and 6-TG/AraC/5-azacytidine in addition to AraC/6-TG and COAP [cyclophosphamide, Oncovin (vincristine), arabinosylcytosine, and prednisone]. The DFCI 80-035 and the SJCRH AML-80 studies both intensified postremission therapy by giving non–cross-resistant chemotherapeutic drugs sequentially over approximately 1 year; they did not achieve significantly better results than prior trials. [290,291] Several studies in adults, however, have strongly suggested that increasing the intensity of immediate postremission therapy, especially with high-dose AraC, resulted in a lower relapse rate and with relapses occurring later than in patients receiving less intense maintenance therapy. [292,293] This was especially true for patients with favorable cytogenetics [e.g., t(8;21) or inv(16)]. [294,295]

The CCG-213 trial from 1985 through 1989 assigned patients to a 4-month postremission intensification treatment and then randomized to 2 years of maintenance therapy or no further treatment.[296] This study and the CCG-213P showed that postremission therapy (intensification) impacted positively on the outcome of patients with AML.[296] Other studies, such as the SJCRH AML-83, BFM-83, BFM-87, and POG-8498, all suggested that increasing the dose intensity, particularly with AraC, resulted in a lower relapse rate and increased remission duration than with more conventional lower dosing of AraC. [270,287,291,297] Recent reports from CCG have also linked the degree of intensification of induction therapy to outcome. [60,263,264] In the CCG-2891 trial, patients received induction therapy with standard-timing DCTER or intensified-timing DCTER with or without G-CSF. A significant survival advantage was observed for the intensified DCTER induction over standard-timing DCTER regardless of the type of postremission therapy received ( Fig. 20-6). These results have demonstrated that the type of remission one achieves significantly impacts on the overall treatment outcome for patients with AML.[263,264] An analysis from Europe of the impact of intensifying AraC during induction and consolidation was not, however, able to show a difference in outcome after autologous or allogeneic transplantation. [298]

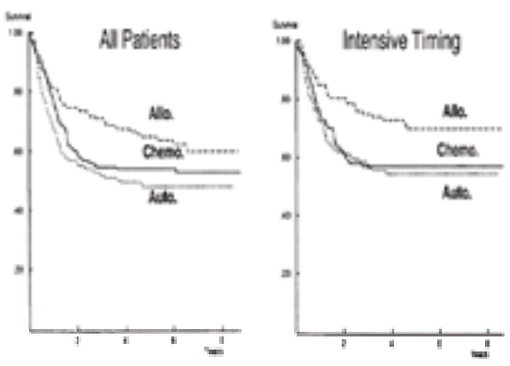


**FIGURE 20-6.** Intensively timed induction therapy results in improved outcome regardless of postremission therapy with allogeneic or autologous transplantation or chemotherapy as reported in the Children's Cancer Group 2891 study.[264] These curves represent numbers for patients going into remission. Please see schema in Figure 20-5. Allo, matched related donor hematopoietic stem cell transplantation; Auto., autologous hematopoietic stem cell transplantation; Chemo., chemotherapy only arm. (Reproduced with permission from W.B. Saunders Company.)

## Postremission Intensification with Hematopoietic Stem Cell Transplantation

### *Allogeneic Hematopoietic Stem Cell Transplantation*

In part because of the continuous improvement in EFS in patients with AML receiving intensified chemotherapy, the potential for further intensification using myeloablative therapy followed by allogeneic or autologous hematopoietic stem cell rescue has been an appealing alternative to treat patients with AML. The potential advantages of allogeneic transplantation include the absence of leukemia in the donor graft as well as a graft-versus-leukemia (GVL) response. Treatment related morbidity and mortality are usually due to direct organ damage as well as the effects of graft-versus-host disease (GVHD) and prolonged immunosuppression. Long-term problems include chronic GVHD, growth problems, sterility, and the risk of secondary malignancies. [244,299,300,301,302,303,304,305,306,307 and 308]

The importance of an immune-mediated antileukemic response has been clearly documented in patients with either CML or AML after allogeneic stem cell transplantation. However, most GVL has been difficult to separate from GVHD. For example, attempts to reduce GVHD by extensive T-lymphocyte depletion from donor grafts have resulted in higher relapse rates, particularly for patients with CML. [309,310] This is also strongly suggested by the higher relapse rates when identical twins are used as donors in contrast to matched sibling donors. [311]

Most studies, including those using randomization and analysis by intent to treat, have shown an improved RFS in patients with AML undergoing allogeneic stem cell transplantation using matched sibling donors. [299,300,301,302,303 and 304,312,313 and 314] However, in several studies the overall survival was not initially found to be improved with allogeneic hematopoietic stem cell transplantation (HSCT) because of increased treatment-related mortality. [295,315] As improvements in supportive care and GVHD prophylaxis measures have been developed, treatment-related mortality has significantly decreased for HLA-matched family donor transplantation, resulting in more studies showing an overall survival advantage for transplantation. [300,312,313,316,317]

The first pediatric study published that used biologic randomization was the SJCRH AML-80 trial. [291] Although the 6-year DFS for patients receiving HLA-matched HSCT was 43%, compared to the 31% for those receiving only chemotherapy, these percentages did not achieve statistical significance. However, when deaths due to treatment-related toxicity were excluded from the analysis, the HSCT group had a 70% DFS at 6 years compared to 38% for the chemotherapy-only group. In the CCG-251 study, which began in 1978, patients receiving an HLA-matched HSCT had a significantly better 5-year EFS than those receiving chemotherapy. [232,318] This advantage appeared to be abrogated in the subsequent CCG-213, in which postremission chemotherapy was further intensified. [319] However, when the results of CCG-213 were analyzed by the therapy the patients actually received rather than by intent to treat, a survival advantage was evident for those patients undergoing HLA-matched HSCT.[232,318,319]

Other pediatric studies have also not consistently shown any overall survival advantage for patients receiving HLA-matched HSCT. [291,320,321 and 322] This has been mostly due to the higher treatment-related mortality associated with HSCT compared to intensive chemotherapy. Results from the Associazione Italiana di Ematologia ed Oncologia Pediatrica (AIEOP), the MRC, POG, and more recent CCG trials have demonstrated that relapse-free and overall survival are greater after HLA-matched HSCT (Table 20-5).[229,232,240,264,323,324 and 325] Another important area that has not been definitively examined is the quality of life (QOL) and financial impact of HLA-matched HSCT versus other types of postremission therapy.[326] Nevertheless, for younger patients, allogeneic transplantation using HLA-matched family donors results in the best overall survival outcome in several large, randomized trials analyzed by intent to treat. This point, however, remains controversial in that some study groups now recommend using HLA-matched family donors and transplantation after a first relapse for patients with favorable characteristics. Whether allogeneic transplantation will achieve at least the same overall outcome advantage when used after relapse remains to be determined, with the exception of patients

with APL (see under Acute Promyelocytic Leukemia).

| Study | DFS | | | Allo versus other (p value) | Chemotherapy versus auto (p value) |
|---|---|---|---|---|---|
| | Chemotherapy (%) | Auto-HSCT (%) | Allo-HSCT (%) | | |
| AIEOP | 21% (37) | 21% (15) | 51% (24) | <.05 | NS |
| CCG-251 | 32% (252) | NS | 65% (89) | <.05 | NA |
| CCG-213 | 38% (298) | NS | 48% (110) | NS | NA |
| SJCRH | 31% (42) | NS | 43% (19) | <.05 | NA |
| CCG-2891 (all patients) | 47% (370) | 42% (177) | 47% (170) | <.05 | NA |
| CCG-2891 (intensive timing) | 53% (168) | 48% (115) | 53% (108) | <.05 | NS |
| LAM-89/91 | 40% (114) | NS | 72% (33) | <.05 | NA |
| POG 8821 | 36% (117) | 38% (115) | 52% (89) | NS | NS |
| MRC AML 10 (BFM REF | 46% (50) | 48% (50) | 61% (85) | NS | <.05 |

AIEOP, Associazione Italiana di Ematologia ed Oncologia Pediatrica; Allo, allogeneic; auto, autologous; CCG, Children's Cancer Group; DFS, disease-free survival; LAM, Leucemia Acuta Meiloide; MRC AML, United Kingdom's Medical Research Council acute myelogenous leukemia study; NA, not applicable; NS, not significantly different; POG, Pediatric Oncology Group; SJCRH, Saint Jude Children's Research Hospital.
Note: Survival with chemotherapy versus auto-HSCT versus allo-HSCT.
*DFS for AIEOP trial is at 5 years; DFS for CCG-2891 study is at 8 years; DFS for AIEOP study is at 5 years; DFS for MRC AML 10 Trial is at 7 years; DFS for POG 8821 is at 3 years.
†MRC AML 10 compared auto-HSCT versus no further chemotherapy. Although fewer relapses were observed in the allo-HSCT group (p = .02), overall survival was not different. In addition, there was no overall survival advantage observed with any of the treatment groups.[60]

**TABLE 20-5. ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) COMPARED TO CHEMOTHERAPY IN CHILDREN WITH ACUTE MYELOGENOUS LEUKEMIA (AML)**

***Autologous Hematopoietic Stem Cell Transplantation***

Attempts to avoid the impact of GVHD but provide stem cell rescue after hematopoietically ablative therapy have included performing autologous transplantation (auto-HSCT) using bone marrow or peripheral blood stem cells.[263,264,327,328,329,330,331,332,333,334,335,336,337] and [338] Several advantages of auto-HSCT include potentially less transplant-related mortality, the lack of acute and chronic GVHD, and the availability of donor cells for most patients. However, the lack of GVL and the potential for returning leukemic cells contaminating the donor bone marrow or peripheral blood stem cells may be significant disadvantages. [339] Genetic marking studies have shown that leukemia cells contaminating bone marrow can contribute to relapse.[340,341] And though *ex vivo* treatment of the autologous stem cell source by MABs or cytotoxic agents has been used, definitive studies that such an approach truly impacts on the extent of relapse are currently needed.

The results for auto-HSCT in adult studies have tended to show an increased RFS compared to nonablative chemotherapy but inferior to HLA-matched HSCT.[299,342,343,344,345,346,347,348,349] and [350] The improvement in survival with auto-HSCT in adults may in part be that intensification can be tolerated with stem cell rescue on a one-time basis, but that sequential, intensified chemotherapy is not as well tolerated. [351] The MRC AML 10 trial showed an advantage for autologous HSCT over no further treatment, but did not directly compare auto-HSCT with another course of chemotherapy.[325]

Several randomized pediatric trials have been completed and analyzed by intent to treat.[60,264,324,325,347] These studies have shown no significant benefit in DFS or EFS for auto-HSCT compared to postremission chemotherapy in these trials. This lack of a significant advantage is in part due to the increased efficacy of intensification for chemotherapy only, but may also be due to the fact that residual leukemia cells are present in the autologous graft as well as to treatment-related mortality during transplantation. The lack of a significant immune-mediated antileukemic response accompanying auto-HSCT or chemotherapy may partly explain the advantage of allogeneic HSCT over these other treatment modalities. Clearly, the ability to generate such responses in the setting of auto-HSCT or postremission chemotherapy would potentially enhance the efficacy of these treatments without the higher incidence of treatment-related morbidity and mortality commonly associated with allogeneic HSCT.

Several conclusions can thus be made in terms of therapy for patients with AML. Dose intensification and supportive care measures have significantly contributed to improved outcomes. Comparison of the most successful approaches to date show that there are several therapeutic strategies that result in similar outcomes as long as significant dose intensification is part of the treatment ( Table 20-6). Although the CCG-2891, LAM-89/91, and the MRC AML 10 studies all use a relatively short duration of therapy, the BFM studies continue to employ longer periods of therapy. In each of these studies, intensification with high-dose AraC appears to an important component. In addition, the escalation of anthracyclines was particularly notable in the MRC AML 10 trial ( Table 20-7). Although the results of the MRC AML 10 trial are excellent, the issue of increasing the cumulative anthracycline dose to such an extent is of concern, especially in very young patients. However, despite dose intensification and the introduction of other chemotherapeutic agents, the primary cause of death in patients with AML remains leukemia.

| Study | Complete remission rate (%) | Patient number | Event-free survival (%) (yr) | Overall survival (%) (yr) |
|---|---|---|---|---|
| AIEOP | 79 | 161 | 25 (5) | 42 (5) |
| EORTC-58872 | 77 | 108 | 41 (3) | 56 (3) |
| LAM-87/91 | 87 | 172 | 47 (4) | NR |
| BFM-87 | 78 | 307 | 43 (5) | 48 (12) |
| POG-8821 | 85 | 649 | 34 (3) | 42 (3) |
| CCG-2891 (intensive timing) | 75 | 652 | 42 (3) | 51 (3); 49 (8) |
| CCG-2891 (standard timing) | 70 | 652 | 27 (3) | 39 (3); 34 (8) |
| MRC AML 10 | 92 | 341 | 49 (5) | 59 (5) |

AIEOP, Associazione Italiana di Ematologia ed Oncologia Pediatrica; BFM, Berlin-Frankfurt-Munster; CCG, Children's Cancer Group; EORTC, European Organization for Research on the Treatment of Cancer; LAM, Leucemia Acuta Meiloide; MRC AML, United Kingdom's Medical Research Council acute myelogenous leukemia study; NR, not reported; POG, Pediatric Oncology Group.

**TABLE 20-6. OVERALL REMISSION RATE AND SURVIVAL IN RECENT PEDIATRIC ACUTE MYELOGENOUS LEUKEMIA TRIALS**

| Study | Anthracycline | Cytarabine |
|---|---|---|
| BFM-87 | 300 mg/m² | 40 g/m² |
| BFM-93 | 400 mg/m² | 44 g/m² |
| POG 8821 | 405 mg/m² | 38 g/m² |
| AEIOP | 545 mg/m² | 17 g/m² |
| LAM-89/91 | 520 mg/m² | 21 g/m² |
| MRC AML 10 | 650 mg/m² | 11 g/m² |
| CCG 2891 | 350 mg/m² | 32 g/m² |

AIEOP, Associazione Italiana di Ematologia ed Oncologia Pediatrica; BFM, Berlin-Frankfurt-Munster; CCG, Children's Cancer Group; LAM, Leucemia Acuta Meiloide; MRC AML, United Kingdom's Medical Research Council acute myelogenous leukemia study; POG, Pediatric Oncology Group.
Note: All values are rounded off to the nearest decimal. Anthracycline amounts are based on doxorubicin equivalents for mitoxantrone or amsacrine.

**TABLE 20-7. COMPARISON OF CHEMOTHERAPEUTIC DOSES IN DIFFERENT PEDIATRIC TRIALS**

Another important lesson from these large clinical trials has been the demonstration that certain subgroups of patients require alternative approaches. For example, outcome is still extremely poor for patients with AML with monosomy 7 or secondary AML.[193,352,353] and [354] Alternatively, patients with DS have improved outcomes with less intense chemotherapy regimens that do not include allogeneic bone marrow transplantation. [59,61,192,193,352,353,354] and [355] Patients with APL have a much improved outcome with the use of ATRA.[356,357] This type of risk group classification is just the beginning of defining subtypes of AML that will be able to be therapeutically targeted based on biologic characteristics.

### Central Nervous System Prophylaxis and Extramedullary Disease

Before the introduction of CNS prophylactic treatment in pediatric patients with AML, the incidence of CNS involvement was reported in the 20% range. [289,358,359 and 360] Most patients who experience a CNS relapse of AML also develop a bone marrow relapse. The highest incidence of CNS leukemia is observed in patients with very high peripheral leukemic blast counts and in M4 or M5 AML subtypes.[358,361] Although pediatric randomized trials have not been performed in terms of patients receiving or not receiving CNS prophylaxis, essentially all treatment protocols or clinical trials include CNS prophylaxis with either intrathecal AraC and methotrexate. The resulting incidence of CNS relapse has been reduced to approximately 5%. [240,263,270,359]

BFM studies have provided an interesting observation on the use of CNS prophylaxis. The BFM-83 study used cranial radiation and intrathecal methotrexate as CNS prophylaxis and observed a similarly low incidence of CNS leukemic relapse. [362] The BFM-87 study attempted to eliminate cranial radiation in low-risk patients by randomizing one group to receive only intrathecal AraC; both groups received high-dose systemic AraC in the postremission period. High-dose AraC treatment results in therapeutic levels of drug in the cerebrospinal fluid. The results showed no significant difference in the incidence of CNS relapses in the two groups, but a higher incidence of systemic incidence in the group of patients who did not receive cranial radiation. [363] These results remain enigmatic, but suggest that the delivery of cranial radiation decreases the chance of later systemic relapse. In contrast to pediatric studies and practice, most adult trials do not include CNS prophylaxis; the incidence of CNS relapse is in the 5% to 10% range, usually associated with systemic relapses.

The occurrence of extramedullary disease in AML usually takes the form of chloromas, which are more commonly observed in patients with M4 and M5 subtypes. Chloromas occur in approximately 10% of patients with AML overall. On occasion, patients may present with an isolated chloroma, but in such situations, systemic disease will become manifest if systemic treatment is not used.[156,164,364] A report from the CCG demonstrated that the EFS was not different for patients who received local radiation treatment to a chloroma in addition to systemic therapy compared to those who received only systemic treatment. In addition, there was no increased incidence of local recurrence at initial sites of chloroma involvement in patients receiving radiation therapy compared to those who did not. [365] In circumstances in which a chloroma may cause significant morbidity, such as loss of vision or spinal cord compression, emergency radiation therapy is usually used. [366,367]

### Acute Promyelocytic Leukemia

APL can be distinguished from other subtypes of AML by virtue of its excellent response and overall outcome as a result of differentiation therapy with ATRA. The PML/RARA fusion protein, resulting from the t(15;17) chromosomal translocation, functions to recruit protein complexes that serve to repress transcriptional activity of different genes. By binding to this fusion protein, ATRA causes a partial release of the repressor complex that results in the expression of genes leading to cell maturation and ultimately apoptosis.[368,369,370 and 371]

When administered as a single oral dose of 45 mg per $m^2$, ATRA achieves peak plasma concentration between 1 to 2 hours with median peak plasma concentrations between 300 to 400 ng per mL.[372,373 and 374] This concentration range is one in which *in vitro* differentiation is induced. The initial trials using ATRA for patients with APL were performed in Shanghai in 1986 with dramatic responses observed.[375] Subsequent studies have confirmed the activity of ATRA in APL patients, with CR rates of greater than 90% being obtained in some studies.[376] CRs achieved with ATRA usually require approximately 5 to 6 weeks of treatment, which is similar to that observed using conventional chemotherapy.[377,378,379,380 and 381] However, remissions obtained using ATRA occur without bone marrow aplasia. Despite the impressive CR rates obtained with ATRA alone, remission duration is relatively short (approximately 3.5 months). [382,383] These results have prompted the use of ATRA followed by postremission chemotherapy, resulting in leukemia-free survival of approximately 71% at 36 months. [379,380 and 381,384,385]

The French APL Group study randomized patients to receive ATRA or chemotherapy with AraC plus daunorubicin. [386,387 and 388] Patients randomized to initial treatment with ATRA received the same chemotherapy when they achieved a CR or earlier for specific levels of leukocytosis. Approximately one-third of patients in the ATRA group received chemotherapy with ATRA initially for WBC greater than or equal to 5,000 per mL and another one-third had chemotherapy added for a rising WBC count. All patients who achieved remission received two consolidation courses of the same chemotherapy. The EFS at 1 year was 83% in the ATRA group compared to 50% in the chemotherapy group ($p$ = .0001). The estimated risk of relapse at 1 year was 13% versus 41% ($p$ = .0006), and survival at 1 year was 91% versus 74% ($p$ = .01) in favor of the ATRA arm.

The North American intergroup trial randomized patients to receive oral ATRA at 45 mg per $m^2$ per day or standard induction chemotherapy with AraC and daunorubicin.[385] All patients who achieved CRs received two courses of consolidation chemotherapy. All patients continuing in remission were then randomized to observation or ATRA maintenance with 45 mg per $m^2$ per day for 1 year. The results from this study showed estimated rates of overall survival at 3 years from entry into the study of 50% for patients who received induction chemotherapy compared to 71% for patients who received induction therapy with ATRA ( $p$ <.001). The overall survival rates by intention to treat showed a similar advantage to the group receiving ATRA, with overall survival being 50% for those assigned to chemotherapy compared to 67% for those assigned to receive ATRA ($p$ <.003).

Two other studies have evaluated induction therapy with the combination of ATRA plus an anthracycline (IDA) without AraC. Estey et al. [389] used ATRA at 45 mg per $m^2$ per day until CR and IDA, 12 mg per $m^2$ per day, on days 5 to 8. CR was achieved in 30 of 39 patients (77%). A larger trial treated 185 patients with untreated APL with ATRA, 45 mg per $m^2$, plus IDA, 12 mg per $m^2$, on days 2, 4, 6, and 8. One hundred fifty-six patients were evaluable for response: ten patients (6%) died within the first week, and the remaining 146 (94%) patients all achieved remission with no cases of resistant leukemia. [390]

Although the introduction of ATRA during induction therapy has not been shown to significantly reduce the risk of severe hemorrhagic complications, the overall induction rate is improved to more than 90%.[385] In addition, maintenance therapy with ATRA has been shown to significantly reduce the incidence of relapse and improve overall survival.[391] These data have thus led to the recommendation of using ATRA during induction and maintenance therapy in the postremission period. [391] Responses and overall results are similar for children and adults, thus showing that in the case of APL, the presence of the t(15;17) translocation is a key therapeutic target. This point is particularly well illustrated by the lack of a significant response in less common variants of APL, including those characterized by the t(11;17) translocation.[146] In addition, some forms of resistance to ATRA may arise from mutations in the PML/RARA fusion protein. [392]

There is also a growing body of data that suggests that the addition of 6-mercaptopurine and methotrexate to ATRA during maintenance therapy may improve overall survival.[393] Additional prospective randomized trials are needed to definitively prove the utility of these other agents in combination with ATRA. Although ATRA provides an important benefit to patients with APL, there are significant side effects that may be associated with its use, some of which, like pseudotumor cerebri, are more frequently encountered in children.[394,395] The acute development of pulmonary edema, also called *ATRA syndrome*, can be a devastating complication that occurs in children and adults. Early diagnosis and treatment with dexamethasone is imperative. [394]

Another important contribution to the treatment of patients with APL has been the introduction of arsenic trioxide, again initially introduced by Chinese investigators. [396] The use of arsenic has been shown to be able to rescue patients who become resistant to ATRA. [397,398] The addition of arsenic to ATRA appears to be synergistic both in an animal model for APL as well as in some patients.[370,397,399] Arsenic trioxide appears to function by inducing differentiation of promyelocytes leading to their death through apoptosis.[370] Randomized clinical trials are ongoing to help determine how best arsenic can be utilized in the treatment of patients with newly diagnosed APL. There are concerns of safety in children exposed to arsenic, particularly to the developing nervous system. Therefore, this promising agent should be carefully studied in children, especially in those who are very young.

### Down Syndrome and Acute Myelogenous Leukemia

Children with DS have an increased risk of developing acute leukemia. Although older studies estimated the excess risk to be three- to 100-fold, more recent studies estimate the risk to be ten- to 20-fold increased.[54,55] Past analysis of leukemia incidence in children with DS have suggested the ratio of lymphoid to myeloid leukemias is approximately 4:1.[54,55] However, a significant number of these cases in children younger than 2 years of age with DS are now known to be due to acute megakaryocytic leukemia (M7 AML), which can be morphologically difficult to distinguish from FAB L2 ALL. In contrast to the rare occurrence of megakaryocytic leukemia in children without DS, M7 AML is now recognized as the most common form of AML in very young children with DS.[400] Overall, children with DS are estimated to have a 400-fold increase in their risk of developing megakaryocytic leukemia. [401]

Children with DS and AML have been shown to have a superior response to AML therapy compared to other children with AML. Initial reports from the CCG reported 20 children with DS and AML treated on CCG studies between 1972 and 1984.[402] These children did not have a worse outcome as measured by EFS ($p$ = .66), survival ($p$ = .17), or RFS ($p$ = .70) in comparison to non-DS children with AML. CCG 251 enrolled 15 children with DS and AML (total enrollment, 508).[233] Patients received doxorubicin (or daunorubicin) (30 mg per $m^2$) for 3 days and AraC (100 mg per $m^2$) for 7 days, with remission achieved in 13 of 15 patients.

CCG reported to the American Society of Clinical Oncology in 1990 results from the CCG-213 study. At that time, 78% of 39 patients with DS were surviving in contrast to 38% of 683 non-DS patients with AML, indicating that DS patients had an improved outcome.[403] The POG reported the results of POG 8821 at the 1992 Societe Internationale d'Oncologic Pediatrique (SIOP) XXIV meeting. Twenty-four children with DS received therapy with daunorubicin, AraC, and 6-TG (DAT), followed by high-dose AraC and subsequently by etoposide and 5-azacytidine. Remission was achieved in 20 of 22 patients. Nineteen of 20 patients were reported to be alive in clinical remission with one death due to infection. The POG subsequently published results of POG 8498, which included 12 patients with DS.[59] Induction therapy again consisted of DAT for two cycles. All 12 patients entered remission, with EFS reported to be 100%. Induction success for DS patients was significantly dependent on treatment intensity in the CCG 2891 study. CR rates for DS patients were 93% and 62% on the standard and intensive timing arms, respectively. The failure to achieve CR with intensive timing induction was a result of treatment-related mortality (33%) rather than persistent disease (5%), whereas the reduction in intensity did not increase CR failure due to disease persistence.

Evaluation of postinduction therapy on the CCG-2891 study produced similar results. For DS patients in CR after induction, postinduction therapy using allogeneic bone marrow transplant resulted in a 67% RFS (at 2 years), whereas the use of high-dose AraC (Capizzi) intensification resulted in 91% RFS at 4 years. This is compared to 86% and 52%, respectively, in the non-DS population. These values reflect RFS from end of induction (EOI). There were 1 (2%) and 12 (5%) toxic deaths among patients with DS and non-DS patients, respectively, during this phase of therapy ( $p$ = .68), indicating the postremission intensification phase was relatively well tolerated by the DS patients. Combined, intensive timing induction and bone marrow transplantation resulted in excessive toxicity such that the 5-year EFS for DS patients was approximately 69%. However, with standard timing induction followed by high-dose AraC, the EFS was 88% for patients with DS at 5 years.

### Transient Myeloproliferative Disorder

TMD is a disorder found in patients with DS during the newborn period. It is characterized by an uncontrolled proliferation of myeloblasts. Evaluation of these blasts has revealed them to be of megakaryocytic origin with varying degrees of differentiation and to be clonal in nature.[404] Information regarding the presentation and natural course of this disease has been based solely on a small series of patients and is limited, with no population-based studies yet performed. This disorder is distinguished from congenital AML primarily by its spontaneous resolution within the first 3 months of life. Recommendations currently are for supportive care only. Hayashi et al.[405] compared DS patients with TMD to those with AML (M7). In this relatively small group of patients, those with TMD had lower blast percentages in their bone marrow than in their peripheral blood. Patients with AML had clonal cytogenetic abnormalities, whereas none were seen in patients with TMD.

TMD may result, however, in significant morbidity and mortality, with some children presenting moribund at the time of birth with severe hydrops fetalis. Organ infiltration, primarily hepatic, may be severe, progressive, and fatal. Of 13 severely affected patients with TMD, reviewed by Zipursky et al.,[406] five were stillborn, and two died later of their disease. Nine of the 13 presented with hydrops fetalis, and four others had hepatosplenomegaly along with pericardial, pleural, and peritoneal effusions. Hydrops in these patients has been ascribed to severe anemia (not often present) or to cardiac dysfunction secondary to tissue infiltration by leukemic blasts, or both. Causes of death also include DIC, renal failure, or hepatic failure, typically occurring in the first few months of life. Although the true incidence of TMD is unknown, Zipursky has estimated the incidence of severe TMD in DS patients to be as high as 10%.[404,407]

It is known that some of these patients go on to later develop acute leukemia (AML or ALL).[408] Lu et al.[409] found that 9 of their 43 (21%) DS patients with M7 AML had a preceding history of TMD. In a review of case reports from the literature, Zipursky identified 7 of 27 (26%) TMD cases who eventually developed acute leukemia. In a subsequent review, 30% of 62 cases of TMD, identified in a questionnaire survey, went on to develop leukemia in the first 3 years of life.[404,410] An important point to keep in mind is that current estimates may be falsely high due to reporting bias. TMD may sometimes require treatment with exchange transfusion or low-dose cytoreductive therapy.

## PROGNOSTIC FACTORS

AML is a collection of related diseases that are heterogeneous in their etiology, pathogenesis, genotype, and phenotype and in response to therapy. Since the 1980s, many studies have attempted to define particular subsets of patients whose treatment could be more precisely targeted to the subtype of AML or to host and disease characteristics. The results from such studies have not been entirely consistent, which may, in part, reflect the manner in which various characteristics are defined, the differing numbers of patients examined in particular studies, as well as differences in the age of patients and types of treatment used. More recently, data obtained concerning cytogenetic and molecular characteristics have started to complement the more traditional variables such as demographics, host characteristics, and disease presentation. It should be kept in mind that prognostic factors are inherently determined in part by the treatment that is used.[411]

### Demographic, Host, and Disease Predictors of Response

Several studies in adults with AML have helped to define several favorable characteristics for patients achieving an initial CR.[412,413 and 414] Some of these characteristics include being younger than 60 years old, absence of secondary AML, a WBC of less than 100,000 per µL, as well as specific chromosomal abnormalities such as t(8;21) or inv(16).[154,193,415,416,417 and 418] In addition, patient characteristics have been defined in terms of their impact on predicting the duration of remission with younger age (younger than 18 years); absence of secondary AML; and chromosomal abnormalities, such as t(8;21), inv(16), and t(15;17), contributing favorably to a longer remission.[413,418,419 and 420] FAB classification has suggested that patients with M1 with Auer rods, M3 or APL, and M4 with eosinophilia have a better outcome.[421,422 and 423]

In the CCG 213 study, adolescents older than age 15 years were shown to have a poor outcome.[320,424] In some of the relatively early trials, infants were reported to respond less well than other children to therapy. For example, in CCG 251 and 213 studies, infants with monoblastic leukemia were shown to have a poorer prognosis than other patients.[320] In addition, infants younger than 1 year of age had a worse outcome than those age 1 to 2 years. In contrast, studies from POG showed that patients younger than age 2 years had a favorable outcome.[297] Of interest, infant leukemia of the M5 FAB type is often associated with translocations involving the 11q23 chromosomal region, which is similar to that observed in podophyllin-induced leukemia.[425] Such secondary AML cases usually have an unfavorable outcome with conventional therapy.

In CCG cross-study analyses, there has been a strong suggestion that non-white patients have a lower induction and EFS rate; this trend achieved significance for patients of Hispanic origin. In CCG 251, 213, and POG 8498, children with DS constitute a group with a significantly better outcome.[59,151,426] CCG 2891 has confirmed this favorable prognosis for DS patients with AML and further demonstrated that less intensive therapy results in an improved outcome.

The role of FAB classification and immunophenotyping with outcome measures has been controversial for pediatric patients.[427,428 and 429] Whereas several studies have shown that monoblastic leukemia portends an unfavorable prognosis, some CCG studies have found that M5 morphology is unfavorable only in infants.[320,421,430] In addition, although most patients with FAB M6 and M7 morphologic subtypes have an overall reduced EFS, young patients with DS and M7 AML have a significantly improved survival.[59,61,431]

Tumor burden and sites of involvement have been shown to correlate with prognosis in multiple studies. In CCG-251 and CCG-213, a WBC of greater than 100,000 per µL was unfavorable.[320] Patients with CNS disease at diagnosis have been reported to have a higher risk of early bone marrow relapse or recurrent CNS disease, or both.[432] Review of CCG patients with isolated chloromas has shown a greater survival compared to those with chloroma and marrow disease. In patients with WBC less than 20,000 per µL, those with chloromas had an improved 5-year EFS compared to others; but for patients with WBCs greater than 20,000 per µL, no difference in 5-year EFS was observed.[365] Radiation therapy did not change the EFS. An important issue to determine will be whether patients with isolated chloroma and low tumor burden may define a unique subset of AML that needs less intensive therapy.

Although chromosomal abnormalities have been reported to correlate with outcome in adults with AML, the same differences have not always been observed in pediatric patients.[415,433] For example, adult patients with t(8;21), inv(16), or t(15;17) (with or without other cytogenetic abnormalities) have been shown to have a

favorable prognosis. An intermediate risk group had AML characterized by trisomy 8, trisomy 21, or t(6;9) with or without other cytogenetic abnormalities. A poor prognosis group has been identified as having AML with t(9;22) or –7 or del(7) or del(11) with or without other cytogenetic abnormalities. [378,415,434]

Results in pediatric trials have not consistently reported a significantly better prognosis for patients with these "favorable" chromosomal abnormalities. SJRCH and the CCG-213 study have reported that patients with t(9;11) and inv(16) abnormalities have an improved outcome. [154,435] Although quite interesting, such results need confirmation in larger, prospective trials. The CCG reported that leukemias with t(8;21), +8, and t(15;17) have improved induction percentages, but that long-term survival was not significantly better. [436] Overt leukemias (in contrast to MDS) with –7 or 7q– show both a reduced remission induction rate as well as an overall worse prognosis of below 20% EFS.[437] In addition, the presence of a t(15;17) translocation clearly defines a group of patients who will significantly benefit by therapies containing ATRA.[437,438 and 439] Studies from POG have demonstrated an improved EFS in patients with inv(16) and t(8;21) of approximately 60% and 45%, respectively, but a worse EFS (approximately 20%) for patients with 11q23 abnormalities. [193] Overall EFS, however, was approximately 35%, which is less than other more recent studies.[193,440] Thus, though cytogenetic analysis may not absolutely define a "very good" prognosis group in pediatric patients with AML, there is considerable evidence that certain chromosomal changes are strongly correlated with extremely poor-prognosis AML.

The prognostic significance of the differentiative state of leukemic blasts has also been extensively examined through the use of cell surface markers, including those representing lineage infidelity such as B- and T-lymphoid antigens. [441,442] Although several studies have claimed that the presence of lymphoid markers on AML blasts represents a poor prognostic variable, this observation has not been unequivocally confirmed. In CCG-251, increased expression of the CD33 myeloid differentiation antigen was associated with a poorer outcome.[436] However, in the subsequent CCG-213, neither CD33 or other surface antigens were found to be of prognostic value. This might have been due to the more aggressive treatment regimen in the CCG-213 trial. [436] Recent CCG studies have identified a leukemia surface antigen, which is recognized by MAB 7.1.[443] The 7.1 MAB has been shown to detect the human homologue of the rat NG2 chondroitin sulfate proteoglycan molecule previously shown to be expressed on human melanoma. The expression of the NG2 molecule in childhood AML was associated with a poor outcome and with some, but not all, cases with 11q23 rearrangements.[418,443] Confirmatory studies should be done to define the expression of this marker with prognosis. The flt-3 receptor, a type III tyrosine kinase receptor, plays an important role in survival and expansion of cells of the myeloid lineage. [444,445 and 446] In approximately 20% to 30% of AML, this receptor is mutated and has an intracytoplasmic tandem duplication or activating single amino acid mutations. These activating mutations have been strongly correlated with a poor outcome in patients with AML.[447,448 and 449]

Since the identification of the multidrug resistance (MDR) P-glycoprotein as a mechanism of chemotherapeutic drug efflux and a cause of tumor cell resistance to specific cytotoxic agents, many studies have examined the expression pattern and potential role of this pathway in AML. [198,199,450] The expression of the MDR P-glycoprotein gene, especially in conjunction with CD34 antigen expression, has been strongly correlated with a poor prognostic group of adult patients with AML.[451,452,453,454,455,456 and 457] Such patients often have relapse or refractory disease or secondary AML. Although both MDR P-glycoprotein and CD34 are expressed on the leukemic blasts in a significant number of pediatric patients with AML, a similar correlation with poor prognosis has not been consistently observed. [458] Although the MDR P-glycoprotein system has been the subject of extensive analysis, it is also clear that other mechanisms of drug resistance function in tumor cells, including those of AML.[198,199,459] Other mechanisms of drug resistance that may have particular importance in AML include the MDR-associated protein, the lung resistance protein, and nucleoside transporters and metabolic pathways. [459,460] Thus, the expression and function of MDR P-glycoprotein and related drug transporters in pediatric AML as prognostic factors or as therapeutic targets remain an important area for further investigation.

More information is also accumulating on the expression and function of a variety of gene products involved in cell proliferation and apoptosis in AML blasts. [93,200] Gene products, such as Ras, are now known to play important roles in the development of AML as well as in the proliferation and survival advantage of AML blasts.[461,462,463 and 464] For example, the importance of Ras is evidenced by the predisposition of patients with NF-1 to develop AML and particularly monosomy 7 and juvenile myelomonocytic leukemia.[465,466,467,468,469 and 470] Patients with NF-1 have a genetic defect in the NF-1 gene that encodes a protein, neurofibromin, which normally accelerates the conversion of active Ras-GTP to inactive Ras-GDP. Decreased neurofibromin activity results in constitutively activated Ras protein, which in turn results in signaling through the MAP kinase pathway leading to cell proliferation. In a mouse NF-1 knock-out model, an increased incidence of myeloid leukemia is observed when the hematopoietic precursors from NF-1 null embryos are transplanted into syngeneic adult animals and particularly after exposure to alkylating agents.[471,472 and 473]

The expression of genes involved in apoptotic pathways has also been examined in leukemia and a variety of other malignancies. [93,200,474] For example, increased expression of bcl-2 has been associated with the decreased ability of a cell to undergo apoptosis and increased resistance to chemotherapeutic or radiation-induced genotoxic damage.[475,476 and 477] The expression and function of other tumor suppressor genes, such as p53, have been correlated with increased resistance to genotoxic agents.[478,479] Several reports have shown that high levels of expression of the Wilms' tumor gene (WT1) is associated with a worse long-term prognosis in AML and that its expression might be used to detect MRD.[480,481 and 482] Genes involved in DNA repair, cell cycle control, proliferation, differentiation, and apoptosis should become increasingly important, not only in terms of diagnosis and risk group stratification, but also as potential therapeutic targets.

Studies in adult and pediatric patients with AML have therefore begun to delineate particularly high-risk, as well as what might be considered better risk, groups. Currently available information has clearly shown that certain groups of patients [e.g., patients with DS and AML or patients with t(15;17) APL] should be treated on separate trials to address unique clinical and biologic features. However, for the remaining pediatric patients with AML, it is much less clear that there is a very good prognosis group that would benefit by decreased therapy. In contrast, other patients may constitute particularly poor prognosis groups, such as AML with monosomy 7, very high WBC at presentation, or secondary AML. Because of small numbers of patients in such subgroups, it may prove very difficult to generate statistical significance for randomized therapeutic comparisons. However, so-called "very high-risk" patients may provide an opportunity for single-arm studies designed to improve outcomes through testing novel therapeutic approaches, including alternative HSCT regimens or targeted cytotoxic agents, as well as further defining the biology of AML subtypes. These studies could also be developed as international trials to accrue larger numbers of patients. Critical to the success of future outcomes will be the close linkage of cellular and biologic studies aimed at identifying risk group characteristics and new therapeutic targets as part of all experimental clinical trials.

**Response to Therapy and Minimal Residual Disease**

The response to initial chemotherapy has proven to play an important role in patients with ALL. [483,484,485 and 486] Although it is likely that the initial response to therapy in AML may also be predictive of outcome, definitive data are currently lacking. The results of intensive timing (e.g., CCG 2891 induction) show that the initial treatment and the resulting quality of remission can predict subsequent outcome. To this end, the response to therapy on day 7 and 14 bone marrow examinations has been reported to predict subsequent outcome.[268] Such data would suggest that level of MRD, both early on during therapy as well at later times, has important consequences for ultimate outcome.

The development of additional methods to accurately detect MRD below the sensitivity level of light microscopy or classical cytogenetic analysis would aid in the ability to test the hypothesis that a certain level of MRD predicts inevitable relapse in AML. The ability to predict patients at highest risk for relapse due to residual leukemia at different stages of therapy would potentially allow alternative treatment interventions.

Several approaches for the detection of MRD have been developed. [114,115,487] The criteria by which such methods are judged must include (a) a strong positive predictive level that is not based just on a statistical probability but is directly related to the presence of residual leukemia; (b) a strong negative predictive value, indicating a high level of specificity and minimizing the number of uncertain results; (c) a high level of applicability so that it can be applied across different subgroups of patients; (d) a rapid turnover of results so that alternative clinical interventions could be made in a timely fashion; (e) cost effectiveness; (f) reproducibility; and (g) availability.[114,115,487] Although no currently available test for MRD perfectly satisfies all these criteria, several methods are available that are likely to be adequate to definitively address the role of MRD in predicting relapse.

The growth characteristics of various AML blasts *in vitro* have been extensively studied, and the outgrowth of colony-forming unit-blast colonies can be observed in agar culture systems. However, this type of approach is methodologically complex and often extremely difficult to quantitatively reproduce. [488,489] Another approach has been to expand residual leukemic cells in immunodeficient mice.[91,92] Several reports have demonstrated that this is possible from relatively small numbers of cells, but these assays are quite laborious, expensive, and the results remain difficult to quantitate. Although the use of FISH methods for the detection of specific chromosomal abnormalities has proven particularly useful, this approach also suffers from not being universally applicable to many patients with AML. [490,491 and 492] In addition, it requires the short-term culture of cells from patients before analysis. The sensitivity of this method to detect specific abnormalities (e.g., trisomy 8) has been

estimated to be approximately $1 \times 10^{-1}$ to $1 \times 10^{-2}$.[114,115,487]

Use of PCR to detect chromosomal rearrangements or resulting chimeric transcripts has provided an extremely sensitive approach to detecting MRD.[114,115,487,493] A disadvantage of this approach, however, is that an informative DNA alteration or transcript must be present in the leukemic blasts. One of the first models developed using this approach has involved detection of the t(9;22) bcr-abl gene fusion associated with CML. Several studies have shown that persistence of the t(9;22) translocation strongly predicts relapse.[494,495,496,497 and 498] There has also been data showing that persistence of the t(15;17) fusion transcript in patients with APL results in a high likelihood of relapse.[499,500,501,502,503,504 and 505] The detection of t(8;21) fusion transcripts has been observed in patients who have been in remission for many years, suggesting that this particular translocation may represent an early event but one not sufficient to produce true leukemia.[506,507,508 and 509] However, a recent report has argued that t(8;21) transcripts do disappear in patients posttreatment who remain in remission.[510] Work on other chromosomal abnormalities, such as inv(16) and 11q23, remains preliminary but may also be complicated by the fact that abnormalities at these locations may be complex and quite heterogeneous, making the application of PCR or RT-PCR methods more difficult.[511,512]

A further important use of PCR or RT-PCR is the detection of mutated genes such as Ras. Mutations in Ras have been observed in greater than 25% of AML in adult and pediatric patients.[496,513,514 and 515] However, though Ras mutations may play a role in the evolution of some cases of AML, it has been observed that relapse samples do not always have the same mutation as in initial diagnostic specimens or may not even show any mutation.[496,513,514 and 515] Thus, because of the apparent instability of Ras mutations as well as their presence in only a percentage of AML, they may not prove to be the best clinical assay for predicting relapse. The expression of WT1 transcripts in leukemia has also been suggested to be potentially useful as a marker for MRD.[481,482,516,517] It has been estimated that approximately 77% of AML cases express WT1 transcripts. However, the FAB subtype of M5 was positive for WT1 expression in only approximately 40% of cases studied.[518,519] Although WT1 is normally expressed at highest levels in the developing kidney, it has also been shown to be transiently expressed during differentiation of normal CD34+ progenitors.[480,481,518] Thus, the expression of WT1 in AML may be in part explained from its transient expression in the normal progenitor compartment. Further work is needed to determine whether WT1 is a useful marker for detecting MRD and predicting relapse for patients with AML.

Another approach being used by several groups is that of multiparameter flow cytometric (MFC) detection of aberrant surface antigen expression on AML blasts.[114,115,487,520,521] MFC is able to simultaneously measure multiple inherent variables, including cell size, granularity, and the expression of several cell surface antigens. By selecting specific sets of parameters, the examination of immature leukemic blast cells can be detected. Using this methodology, it has been estimated that the detection of leukemic phenotypes can be achieved at a sensitivity of approximately $10^{-3}$ when at least 30,000 nucleated cells are counted. Several studies have demonstrated that up to approximately 85% of patients with AML have leukemic blasts with characteristics that are able to be distinguished from normal progenitors by MFC. One potential problem with MFC analysis of residual disease is the possibility of alterations of specific antigen expression patterns, which could give rise to false-negative results.

MFC detection of MRD in AML may be a practical means by which to detect MRD. Wormann et al.[522] used MFC in 45 adult patients with AML and were able to detect residual leukemic cells (defined as greater than 0.5% of aberrant antigen expressing cells) after morphologic remission in approximately two-thirds of the patients, with more than one-half of that group showing relapse within 1 year. A retrospective study of children with AML examined a total of 205 serial bone marrow specimens for the presence of residual leukemia in 39 children who had achieved morphologic remission during their course of treatment for AML and compared these results with the subsequent occurrence of relapse. MFC analyses were performed on bone marrow specimens collected at diagnosis, at the EOI, before and after consolidation, and at the end of treatment.[115,487] At the EOI, leukemic cells manifesting an aberrant constellation of surface antigens were identified in the majority of patients (64%) that achieved morphologic remission. Of the 17 patients with residual leukemia that did not receive an allogeneic bone marrow transplant during first CR, all experienced relapse (median, less than 6 months) with the exception of a child with DS. For seven patients with residual leukemia who received a bone marrow transplant, only one relapsed. Evaluation of multiple samples obtained during remission using a time-dependent Cox regression analysis, which controlled for age, sex, morphologic classification, and WBC at diagnosis, showed an estimated risk of relapse during MFC-positive intervals to be 2.8 times greater than during MFC-negative intervals (95%; confidence interval 1.1 to 7.0; $p = .02$). Another study of MRD using MFC has, in addition, shown that that the presence of MRD at the EOI or consolidation was highly correlated with an MDR P-glycoprotein phenotype and relapse, even after an auto-HSCT.[521] Based on such results, future translational research plans should be to further test the role of MFC in detecting MRD at specific time points during the course of therapy and determining the predictive value of this approach.

Although prognostic variables may thus change with the effectiveness of treatment, they can be useful in the identification of patients who can be stratified to specific targeted therapies at diagnosis or, potentially, at different times during treatment. The importance of future genomic-based methods to stratify patients according to predictive molecular features should offer even further capabilities for prognostication and risk-directed therapy.

## TREATMENT OF PATIENTS WITH REFRACTORY/RELAPSED ACUTE MYELOGENOUS LEUKEMIA

Until therapeutic regimens are significantly improved in patients with newly diagnosed AML, the systematic development and testing of new strategies in patients with relapsed or treatment-refractory AML, or both, will continue to be critical. This is particularly relevant in that whereas 75% to 85% of patients with newly diagnosed AML initially achieve a remission, less than 50% are long-term survivors.[523,524,525,526,527 and 528] Patients with relapsed or refractory AML usually respond less well and for shorter duration to reinduction therapies.[336] This clearly relates to the induction of drug-resistant mechanisms and is, in part, related to specific chromosomal abnormalities and the duration of the first remission. The long-term survival for such patients is poor and considered to be less than 20%.[523,529] Outcome in part depends on the time to relapse. For example, patients who relapse on therapy, take a longer time to achieving remission (longer than 50 days), or experience a CR of less than 6 months from diagnosis, have a poor prognosis, with survival at 5 years of less than 20%.[336,523] However, patients who have a CR of greater than a year, may have a 30% to 40% survival at 5 years.[336,523]

Although there is no standard therapy for patients who have relapsed or refractory AML, or both, most studies have demonstrated that the use of high-dose AraC-containing regimens have significant activity at inducing a second remission, even in patients who have previously received this agent at high doses.[525,527,530,531] Frequently, high-dose AraC is used in combination with other agents such as mitoxantrone, etoposide, fludarabine, or 2-chlorodeoxyadenosine.[531,532,533,534,535,536,537,538,539,540 and 541] In many cases, such as with fludarabine and 2-chlorodeoxyadenosine, the combination has been shown to produce synergistic effects.[542,543,544,545 and 546] When used with G-CSF, the fludarabine plus high-dose AraC combination is referred to as the *FLAG regimen* and has been shown to be a very active regimen at inducing remissions in patients with relapsed AML, with up to 70% success rate for patients with a CR1 of greater than 1 year.[536,537 and 538,540] Other data suggest that the addition of IDA to the FLAG regimen (Ida-FLAG) may produce more durable remissions, although significant toxicity may be experienced.[534,538,539,547] Similar results have been obtained with the use of sequential mitoxantrone and high-dose AraC.[525,548] The use of etoposide along with 2-chlorodeoxyadenosine has also been shown to have activity, particularly in monoblastic subtypes.[549] Several single agents have also been tested in patients with relapsed or refractory AML, or both, but the overall response rates and survival (15% and zero, respectively, at 5 years) are significantly lower than that seen with combination therapy (36% and 3% at 5 years).[550,551]

Patients with CNS relapse nearly always develop evidence for bone marrow or systemic disease. Treatment therefore should involve up to six courses of intrathecal chemotherapy (methotrexate or AraC, or both) followed by intensive chemotherapy. Most of these patients then go on to a hematopoietic stem cell transplant and often receive craniospinal irradiation.

In light of the poor results from the use of chemotherapy alone in patients with relapsed or refractory AML, or both, the use of myeloablative allogeneic transplantation is used whenever possible.[552] The type of preparative regimen has not been established.[553] Some centers use combinations of ablative chemotherapy and others use chemotherapy plus total body radiation.[316,554,555,556,557,558,559 and 560] Donors who are HLA matched (or with one antigen mismatches) provide for the best chances of engraftment and least chance of developing significant GVHD.[561,562 and 563] However, in the setting of relapsed AML, alternative sources of hematopoietic stem cell donors are commonly used, including bone marrow or blood as well as cord blood–matched unrelated donors.[564,565,566,567 and 568] By expanding the criteria for an acceptable donor, the majority of patients can usually be offered an allogeneic transplant.[569,570,571,572 and 573] In addition, the use of haploidentical donors provides a potential source of hematopoietic stem cells for essentially all patients.[574,575,576 and 577] As the HLA disparity increases between donor and host, the complications of treatment-related morbidity and mortality also increase significantly, although children appear to be able to tolerate HLA mismatching better than adults.[578,579] In most studies, treatment-related mortality may range from 30% to 50%.[562,571,573,580,581]

Although cord blood donor sources appear to have significantly reduced the risk of GVHD, there exist problems with engraftment as well as the lack of donor

lymphocyte availability for subsequent use to augment GVL effects.[582,583,584,585,586,587,588,589 and 590] The use of matched unrelated donors may result in a higher risk of GVHD, but a lower incidence of nonengraftment as well as a potential source of donor lymphocytes for subsequent use.[591] Haploidentical donor transplants have a significantly higher risk of severe GVHD and nonengraftment.[592] Although these problems can in part be overcome with T-cell depletion of the graft and the administration of immunosuppressive regimens, the risk of severe immunodeficiency accompanied by fatal opportunistic infection or lymphoproliferative disease increase substantially.[593,594]

In spite of the problems associated with the use of allogeneic transplantation, this treatment modality can be effective for patients with relapsed AML, with DFS figures ranging from 20% to 50% for patients with AML, but considerably higher for patients with APL.[569,570,571,572 and 573] The results of hematopoietic transplantation for patients with APL in CR2 are closer to 70% survival.[595,596 and 597] Such results, in combination with the excellent outcome with chemotherapy plus ATRA in newly diagnosed patients, have led to the recommendation not to perform allogeneic transplantation for patients in CR1 with APL.

Another approach to avoiding some of the risks of allogeneic transplantation for patients with relapsed AML is to use autologous transplantation. As noted previously, although this approach has the advantage of not inducing significant GVHD, host cells do not offer a GVL effect either unless possible immunostimulatory approaches are used posttransplantation.[328,598,599 and 600] In addition, the potential for re-infusing leukemia cells with the graft is a proven risk and potentially exists even with attempts at purging leukemia cells.[328,601,602] Nevertheless, some trials have shown significant DFS in selected patients.[556,603,604 and 605]

There is thus a tremendous need for improved therapies for patients with refractory or relapsed AML. The long-term survival for the majority of patients with relapsed or refractory AML, or both, remains poor. This is primarily due to mechanisms of resistance to conventional chemotherapeutic-based treatments. Future challenges will be to develop methods to overcome these drug resistance mechanisms using strategies to inhibit resistance pathways or by the use of novel approaches that circumvent them completely.

## DRUG RESISTANCE MECHANISMS AND FUTURE THERAPIES

Drug resistance can be due to both pharmacologic and tumor cell mechanisms. Important pharmacologic factors contributing to treatment efficacy include drug dose, drug metabolism, and route of delivery.[198,199,606,607,608 and 609] Cellular factors can include (a) changes in drug uptake or drug efflux across tumor cell membrane or between cytoplasm and nucleus (transport mediated resistance), (b) changes in activation or inactivation of drugs within the tumor cell (metabolic resistance), (c) changes in targeted enzymes through altered levels of those targets within tumor cells or through altered affinity of cellular enzymes for the drug (target resistance), (d) changes in DNA repair processes, and (e) changes in the ability of tumor cells to execute programmed cell death or apoptotic mechanisms.[199]

Attempts to inhibit known cellular mechanisms of resistance have met with mixed success. For example, inhibition of the MDR P-glycoprotein drug efflux pump has produced equivocal results in some studies and some advantage in others in patients with AML.[199,607,610,611,612,613,614,615 and 616] In part, this may be due to the dose adjustments made to account for pharmacologic changes that the inhibitors produced as well as levels and type of the inhibitors used. Furthermore, it has become clear that there are multiple drug transporters that are likely not to be equally blocked by a specific inhibitor.[198] Thus, more selective reversal agents based on the expression of functional drug transporters in a patient's leukemia cells may be a useful approach for testing specific types of drug transporter–mediated resistance.[198,199,617]

Another powerful approach to therapy in leukemia takes advantage of MAB or ligand recognition of leukemia or hematopoietic specific surface antigens or receptors.[618] In AML, the primary targets thus far being tested include the CD33 lineage restricted differentiation antigen as well as the GM-CSF receptor.[618,619,620,621,622,623,624,625,626 and 627] In addition, the CD45 antigen has been targeted with radiolabeled MABs with the intent of delivering significant local radiation to the bone marrow while reducing the amount and, thereby, the toxicity of external beam radiation, during bone marrow transplantation.[622,628,629]

The use of the targeted immunotherapy with anti-CD33 MABs, alone or coupled with toxins or radioactive compounds, has proven to be effective therapy at inducing remission in approximately one-third of patients. For example, early phase studies with anti–CD33-calicheamicin immunoconjugates have demonstrated an approximately 37% remission rate in adults with relapsed AML.[618,627,630] A primary advantage of such targeted therapy is that it is directed at cells expressing a lineage-restricted antigen and thus avoids some complications such as mucositis. Thus, although significant periods of neutropenia and thrombocytopenia result from the use of anti–CD33-calicheamicin due to expression on normal hematopoietic progenitors, neutropenia without mucositis appears to be less morbid.[630] However, a significant incidence of hepatic damage, manifested usually by a transient transaminitis, is observed in approximately 25% of patients.[630] Occasional patients have experienced a clinically severe syndrome consistent with veno-occlusive disease. The etiology of the liver toxicity is unclear but may be due to leukemic cell killing in the liver, expression of CD33 on Kupffer cells, or the low level of free calicheamicin observed after the administration of the conjugate. Of additional interest has been the sometimes prolonged time to platelet recovery, resulting in a response criteria of remission without full platelet count recovery.[618,630] It also remains of interest why two-thirds of patients do not respond to this agent, as well as how to use it in conjunction with chemotherapy.

Other mechanisms of drug resistance may result from activation of specific anti-apoptotic pathways. These survival and resistance pathways may be induced as a result of alterations or mutations in genes encoding cytokine receptors (e.g., flt-3 receptor or c-Kit), signal transduction molecules (e.g., Ras), proteins involved in cell cycle progression and/or DNA repair (e.g., p53), or transcription factors (e.g., PML/RARA). Such changes can in turn lead to altered expression of additional anti-apoptotic pathways as in the increased expression of bcl-$X_L$.[631] The development of promising approaches to target such mutant receptors and signal transduction pathways are being actively pursued. For example, STI-571, a small molecule inhibitor of the bcr-abl tyrosine kinase found in CML, also has activity against other tyrosine kinase receptors such as c-Kit and platelet-derived growth factor.[632,633] Clinical trials with this agent are being performed in patients with relapsed AML. Additional compounds that more specifically target receptor mutations or downstream signal transduction pathways are also being developed.[634,635]

The proto-oncogene product, Ras, is mutated in approximately one-third of AML, and Ras-regulated signal transduction is known to play an important part in AML cell growth.[76] Studies have shown that for Ras to function, it must undergo a posttranslational modification, called *farnesylation*, which allows it to be attached to the inner cell membrane. The development of farnesyltransferase inhibitors that can be taken orally and achieve pharmacologically active blood levels have provided the grounds for the further development of this class of compounds in patients with AML.[636,637,638,639,640 and 641] Another interesting approach that is being tested in early phase trials is the use of antisense oligonucleotide inhibitors of bcl-2, a potent anti-apoptotic protein.[642,643,644 and 645]

The mechanisms by which chromatin structure and DNA modification alters gene transcription and subsequent pathways leading to leukemia and, likely, drug resistance, are just now being elucidated. For example, the role of histone acetylation and deacetylation have been demonstrated to play critical roles in transcription by alteration of the nucleosomal contacts with DNA.[646,647 and 648] In addition, the proteins involved in histone deacetylation, called *HDACS*, have been shown to be physically associated with proteins involved in DNA methylation. Both histone deacetylation and DNA methylation contribute to transcriptional repression.[646] In part, the success of ATRA has been its ability to relieve the recruitment by the PML/RARA fusion protein of repression complexes and, thus, allow for the induction of gene expression, leading to promyelocyte maturation.[368,649,650] Other approaches that are being tested in patients involve the use of HDAC inhibitors (e.g., phenylbutyrate) and compounds that inhibit methylation (e.g., azacitidine). The goal of these clinical trials is to induce differentiation followed by apoptosis of AML blasts. An additional benefit of differentiation approaches to treatment may be the induction of surface molecules on leukemic cells that may contribute to immune recognition, stimulation, and subsequent killing.[651]

Although the stimulation of antileukemic immune responses has been the hope of immunologists and oncologists for many years, previous attempts have been compromised by inconsistent methods and responses. However, more recent studies have begun to establish a more scientific basis for developing immunostimulatory vaccine approaches for the treatment of cancer.[652,653]

For example, experimental studies have demonstrated that the transfer into and expression of a wide variety of cytokine genes can result in the immunologic rejection of genetically modified tumor cells in murine models and protection from subsequent tumor cell challenges.[654,655] Although the exact mechanism(s) responsible for this effect generated by different cytokines, particularly GM-CSF, is still uncertain, it is likely to involve the stimulation and recruitment of dendritic cells and their subsequent processing of tumor antigens from dying cells and stimulation of both helper and cytotoxic T lymphocytes.[654,655,656,657 and 658]

Other studies have shown that while tumor cells may express proteins that could serve as potential immune-mediated targets, tumor cells (including most AML cells) do not express critical costimulatory receptors required for appropriate T lymphocyte activation.[652,659,660] Furthermore, if T lymphocytes are allowed to recognize

antigen in the absence of costimulation, then they develop anergy or tolerance to that antigen. [652,659,660] Thus, leukemic blasts may not only escape immune destruction by their lack of costimulatory receptors, they may also tolerize immune effector cells. [652,659,660 and 661] This has been demonstrated to be the case in experiments using murine models of AML, in which AML cells have been transduced with complementary DNAs encoding costimulatory receptors. [656,662,663 and 664] When AML cells are forced to express costimulatory receptors, they are able to generate significant antileukemic cell immune responses, even in animals with established leukemia.[656,662,663 and 664] However, in animals with advanced leukemia (i.e., a large leukemic burden) cytoreduction with chemotherapy followed by a recovery period was required for this vaccine approach to be curative. [665] This corroborates the observation that therapeutic vaccine approaches as well as immunostimulatory approaches using cytokines, such as interleukin-2 or GM-CSF, are likely to be most effective in the MRD setting. [657,666,667,668,669,670,671,672 and 673]

To this end, an ideal minimal disease setting would be following bone marrow transplantation. However, the posttransplantation period is characterized by an often profound immune deficiency with either autologous or allogeneic donor approaches. [674] However, recent experiments in murine systems demonstrate posttransplant periods when immunostimulation by vaccines or cytokines, or both, can be effective at generating antitumor responses. [675] Several clinical trials in patients with AML after autologous or allogeneic transplantation are currently testing this possibility. [600,670,676]

An alternative approach that is being tested involves the *ex vivo* stimulation of T lymphocytes with host leukemia cells and then generated in tissue culture antileukemic cell clones that can be reinfused into the patient. [666,676,677,678 and 679] Although this approach has been feasible and effective in murine models, it has not been shown to be consistently effective in patients. Part of the lack of this approach's effectiveness may be due to the behavior of leukemic cells that allows them to change the expression of immunostimulatory antigens, express immune inhibitory receptors, as well as secrete immunosuppressive substances such as tumor necrosis factor-alpha and FAS ligand. [680,681 and 682]

The use of allogeneic transplantation from major histocompatibility complex–matched family donors for the treatment of AML has proven to be particularly effective, especially in younger patients. This is in part due to a GVL reaction, which, however, is usually impossible to dissociate from GVHD. [683,684,685,686,687,688 and 689] In addition, while complications from GVHD or the immunosuppressive therapy to prevent or limit the outcomes that can be achieved with matched family donors, such complications are even greater with matched or mismatched unrelated donors.[690,691,692,693,694 and 695] Thus, a significant challenge for transplantation is to be able to induce tolerance to normal tissues while preserving a GVL response. This has been achieved in some murine models with the blockade of costimulatory receptors during antigen recognition or with the use of specific cocktails of cytokines that can induce an anergic state in T lymphocytes. [696] The approach of costimulatory receptor blockage in the presence of normal host cells has been attempted in patients with high-risk leukemia in relapse. [697] Although it is too early to assess effectiveness, these studies have shown the feasibility of such an approach in patients. Another important alternative that is being tested is the use of nonmyeloablative allogeneic transplantation with posttransplantation treatment designed to enhance the development of tolerance to host antigens on the part of donor cells.[698,699]

As we approach the future of therapy for patients with AML, there is considerable hope that less toxic and more effective therapies will soon be a reality and not merely an unrealized hope. Instead of dose-intensified combination chemotherapy followed by myeloablative therapy with transplantation, one can anticipate that future protocols for patients with AML will be quite different. They will include genomic expression profiles that help direct lineage-specific differentiation therapies. Cytoreductive therapies will involve nonimmunosuppressive and nongenotoxic inhibitors of signal transduction as well as targeted MAB or ligand conjugates. After the achievement of a minimal leukemia state, either a nonmyeloablative transplant or autologous vaccine may then be used to eliminate residual disease or limit the expansion of leukemic blasts through maintaining antileukemic cell immunity.

## QUALITY OF LIFE AND SURVIVORSHIP

The judgment of whether therapies can be considered successful will depend on not just eradication of leukemia and normal hematopoietic recovery, but also on the QOL that survivors experience. A growing number of studies have documented an increasing number of treatment-related late effects for patients with cancer.[305,306,700,701 and 702] Several of these studies have focused on patients after HSCT and have documented problems with growth, fertility, cardiopulmonary function, psychosocial adaptation, and secondary malignancies. [702,703,704,705,706,707,708 and 709] However, other data demonstrate that some factors, such as cardiac abnormalities, may not be related to complications of transplantation but more on the cumulative dose of anthracyclines. [702,708,710] This is particularly relevant in young children. Recent studies in adults with AML have examined several measures of QOL after bone marrow transplantation versus high-dose AraC-based postremission therapy.[304,711,712,713 and 714] No significant differences were observed between the two treatment groups.

The true clinical effectiveness of a treatment ultimately also includes measures of cost accountability, resource utilization, and impact. Because health care resources are not limitless and have become more carefully scrutinized, an additional important component of any successful treatment program will be how effective it is in terms of both the cost of the actual treatment but also the impact associated with productive life years saved. The ability to accurately assess the cost of care has been examined in several studies.[304,326,715,716,717,718,719 and 720] As non-HSCT treatments have been intensified, requiring prolonged periods of neutropenia as well as blood product and hematopoietic growth factor support, the obvious differences between allogeneic HSCT, auto-HSCT, and intense postremission chemotherapy have diminished. The introduction of ATRA for patients with APL has been shown to significantly reduce the cost of induction therapy. [717] Furthermore, the initial effectiveness of any therapy has been shown to have a great impact on the eventual cost of care in that the treatment of patients in relapse adds considerably to cost and life years lost.

Improvements in the cure rate of children with AML and the reduction of long-term sequelae should ultimately have a major impact on the quality and cost associated with the diagnosis, treatment, and outcome for these patients. This will also hopefully translate into increased use of these approaches throughout the world, with a result being improved outcome for all children and adolescents with AML.

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# 21

# CHRONIC LEUKEMIAS OF CHILDHOOD

ARNOLD J. ALTMAN



## INTRODUCTION

Chronic leukemias are myeloproliferative disorders characterized by a predominance of relatively mature cells. In contrast to the acute leukemias, these diseases are indolent, with a natural history usually spanning several years. Some subtypes, however, may have a rapidly progressive clinical course.

Chronic leukemias are rare in childhood. The most common type, chronic myelocytic leukemia (CML), accounts for less than 5% of all childhood leukemias. Other chronic leukemias discussed in this chapter include juvenile myelomonocytic leukemia (JMML; formerly known as *juvenile CML*), familial CML, chronic myelomonocytic leukemia (CMML), and chronic lymphocytic leukemia (CLL).

## CHRONIC MYELOCYTIC LEUKEMIA

CML is a clonal panmyelopathy involving all the hemic lineages and at least some of the lymphoid lines. It is characterized by myeloid hyperplasia of the bone marrow, extramedullary hematopoiesis, expansion of the total body granulocyte pool, elevation of the leukocyte count (with appearance of the complete range of granulocyte precursor cells in the peripheral blood), and a specific cytogenetic marker, the Philadelphia (Ph $^{1}$) chromosome.

### Historical Background

CML was the first form of leukemia to be recognized as a distinct clinical entity. Donné [1] described the characteristic hematologic changes in 1844; in 1845, Bennett, [2] Craigie,[3] and Virchow[4] independently described the clinical features and autopsy findings. These early observers were impressed by the marked splenic enlargement and peculiar changes in the color and consistency of the blood. On microscopic examination, the blood contained a predominance of colorless corpuscles similar to those found in small numbers in normal blood and in large numbers in pus. Although he could find no focus of inflammation, Bennett attributed the hematologic findings to “the presence of purulent matter.” Virchow used the descriptive term “white blood,” which, translated into Greek, became “leukemia.” Virchow subsequently subdivided leukemia into two categories: splenic and lymphatic.

In 1870, Neumann[5] suggested that the bone marrow, rather than the spleen, was the source of the excess colorless corpuscles in splenic leukemia; subsequent authors referred to splenic leukemia as *myeloid leukemia*. In 1889, Ebstein[6] recognized the clinical distinction between acute and chronic leukemias, and in 1891, Ehrlich[7] introduced techniques for staining blood cells that permitted the morphologic distinction between myeloid and lymphoid leukemias.

The earliest form of therapy for CML was the use of potassium arsenate (Fowler's solution) by Lissauer in 1865 [8]; this treatment produced limited and temporary improvement. Radiotherapy, introduced by Pusey in 1902,[9] produced better and more predictable effects with much less toxicity and became the standard therapy until the introduction of busulfan in 1953. The cytogenetic hallmark of CML, the Ph $^{1}$ chromosome, was described by Nowell and Hungerford in 1960.[10] This was also the first specific chromosomal abnormality associated with a human malignancy, and its discovery inaugurated the era of cancer cytogenetics.

### Epidemiology

CML is primarily a disease of middle age; the peak incidence is in the fourth and fifth decades. Although CML has been diagnosed in infants as young as 3 months (author's personal experience), more than 80% of pediatric cases of CML are diagnosed after age 4 years and 60% after age 6 years. [11,12] No significant racial or sexual predilection exists, and no hereditary component is demonstrable.

The only environmental factor implicated in the etiology of CML is ionizing radiation. An increased incidence of CML has been reported in radiologists, in survivors of atomic bomb explosions, and in persons exposed to therapeutic radiation for treatment of ankylosing spondylitis and other disorders. However, only 5% to 7% of all patients with CML have documented exposure to excessive radiation, and radiation is rarely implicated in pediatric CML. No infectious agent has been related to the pathogenesis of CML. Investigators have estimated that an average of 8 years is required between the original mutational event and the development of clinical symptoms.

### Cytogenetics

The cytogenetic hallmark of CML is the Ph $^{1}$ chromosome. Initially described as a truncated chromosome 22 (22q–), this anomaly is now recognized to result from the reciprocal translocation t(9;22) (q34;q11) ( Fig. 21-1). Breakpoints on chromosome 9 involve the *c-abl* gene and can vary widely (i.e., greater than 100 kb from case to case); on the other hand, breakpoints on chromosome 22 are virtually always restricted to a small (5.8 kb) segment of DNA known as *the major breakpoint cluster region (*bcr*)*. As a result of the reciprocal 9;22 translocation, two hybrid genes are formed: *bcr/abl* on 22q– and *abl/bcr* on 9q+. Although both of these genes are transcribed, *bcr/abl* appears to have the major role in the pathogenesis of CML.

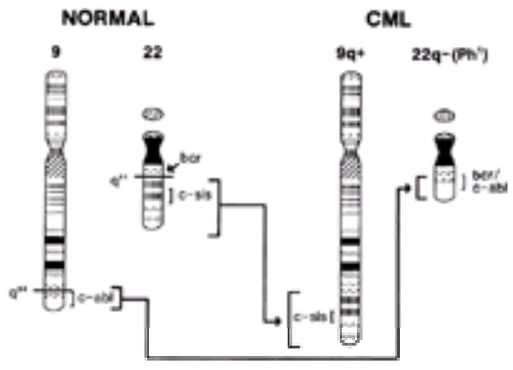


**FIGURE 21-1.** Anatomy of the (9;22)(q34;q11) translocation with formation of the Philadelphia (Ph [1]) chromosome (22q–) containing the hybrid *bcr*/*c-abl* gene. (See text for further explanation.) CML, chronic myelocytic leukemia.

The development of the hybrid *bcr/abl* gene may be a more common phenomenon than is commonly recognized. When studied using a very sensitive reverse transcriptase-polymerase chain reaction (RT-PCR) screening technique, 20% of "normal" adult subjects were found to harbor this translocation. [13] The age distribution of these *bcr/abl*-positive, but otherwise normal, individuals roughly mirrors the age distribution of CML.

Heterochromatin polymorphism patterns indicate an apparent parental bias in the genesis of t(9;22)—that is, the Ph [1] (22q–) chromosome is consistently of maternal origin, whereas the derivative 9q+ is of paternal derivation. [14] If this phenomenon were due to genomic imprinting, the *abl* component of the *bcr/abl* gene would be expected to show an equivalent parental bias; however, molecular analysis indicates that this moiety has an even chance of being the paternal or the maternal copy. [15] These findings may indicate that homologous recombination between 9q22 and 9q34 may be a frequent event and may play a role in the early stages of CML. [16,17]

### *Properties of* c-abl *and Its Protein Product (P145)*

The wild-type *c-abl* gene is the human homolog of the Abelson B cell murine leukemia virus oncogene (v- *abl*) and is universally active in hematopoietic cells at all stages of differentiation. The *c-abl* gene encodes a 145-kd protein (P145) that localizes predominantly in the nucleus. P145 is a multifunctional enzyme that participates in signal transduction and regulation of gene transcription. One major function is to catalyze the attachment of phosphate groups to the tyrosine residues of various proteins [i.e., to act as a tyrosine kinase (TK)]. Members of the TK family are frequently involved in the pathways that transmit signals from the external milieu to the cytoplasm and nucleus; in this capacity, they may act as growth factors, transmembrane receptors, or submembrane catalytic subunits of surface receptors. Under normal circumstances, the P145 TK acts as a negative regulator of cell growth [18]; thus *c-abl* may be regarded as a tumor suppressor gene. As discussed in the section Properties of bcr/abl and Its Protien Product (P210), modifications in *c-abl* may disrupt cell cycle control and result in oncogenesis.

In addition to its TK activity, P145 contains other functional domains ( Table 21-1). The carboxy-terminal portion of P145 contains a domain involved in binding to F actin as well as a separate domain that can bind to DNA; the DNA-binding activity appears to be cell-cycle regulated by the cyclin-activated cdc-2 kinase. At the N-terminal region of P145 (Fig. 21-2) are three domains known as *src-homology (SH) regions* because of their kinship to the viral src oncogene. The first domain is SH1, which manifests weak tyrosine-kinase activity and appears to be tightly regulated by the SH2 and SH3 regions. The presence of SH2 and SH3 regions is significant because these domains play critical roles in intermolecular interactions that specifically mediate protein-protein coupling. The second domain, the SH2 region, can bind to substrates that are tyrosine phosphorylated, whereas the SH3 domain complexes with proline-rich regions involved in coordinating cytoskeletal interactions. Proteins containing SH2 and SH3 domains are classified as adapter molecules because they couple nonreceptor TKs to downstream signaling cascades regulating gene expression.

| Protein | Chromosome | Domain | Function |
|---|---|---|---|
| p145[c-abl] | 9q34 | N-terminal | |
| | | SH1 | Tyrosine kinase |
| | | SH2 | Interacts with tyrosine-phosphorylated proteins |
| | | SH3 | Suppression of tyrosine kinase activity |
| | | Myristylation site | Localization of p145[c-abl] to nucleus |
| | | C-terminal | DNA binding, nuclear localization, actin binding |
| p160[bcr] | 22q11 | N-terminal | |
| | | Coiled-coil motif | Polymerization with other proteins |
| | | Catalytic domain | Serine-threonine kinase |
| | | Central | |
| | | GEF | GDP-GTP exchange factor |
| | | C-terminal | |
| | | GAP | GTPase activating protein for 1HC and 1HC1/G25-related proteins |
| p210[bcr/abl] | t (9;22)(q34;q11) (Ph[1] chromosome) | BCR | |
| | | Coiled-coil motif | Increase tyrosine kinase activity of ABL |
| | | | Enables binding of F-actin to ABL |
| | | Tyrosine residue 177 | Docking site for adaptor proteins |
| | | Serine-threonine kinase | Activation of signal-transduction proteins |
| | | ABL | |
| | | SH1 domain | Phosphorylation of signal and adaptor proteins |
| | | Actin-binding domain | Cytoplasmic location. Interference with adhesion |

GAP, guanosine triphosphate–activating protein; GDP, guanosine diphosphate; GEF, guanosine nucleotide exchange factor; GTP, guanosine triphosphate.

**TABLE 21-1. FUNCTIONAL DOMAIN OF ABL, BCR, AND BCR/ABL PROTEINS**

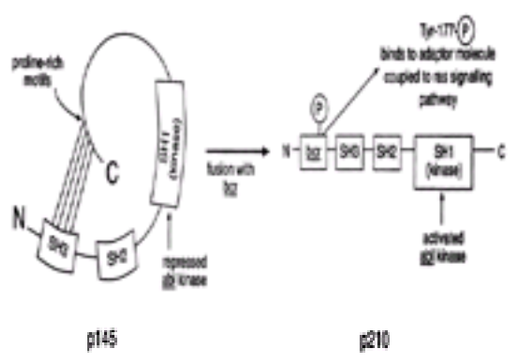


**FIGURE 21-2.** The normal abl tyrosine kinase (TK) component of P145 is carefully regulated by the SH2 and SH3 domains; genetic alterations that prevent interaction of the SH3 domain with proline-rich motifs within the C-terminus result in derepression (constitutive activation) of the TK. In Philadelphia chromosome–positive chronic myelocytic leukemia, fusion with the bcr gene leads to production of a chimeric protein (P210) with activated TK and oncogenic properties. Two domains within bcr are required for its oncogenic effect: domain I mediates oligomerization of *bcr/abl* and promotes phosphorylation of tyrosine residue 177 within domain II, which, in turn, binds to a signaling adaptor molecule coupling *bcr/abl* with the ras signaling pathway. (See text for further explanation.)

### *Properties of* bcr

The *bcr* region is a component of a much larger gene known as *BCR*. BCR has at least three domains (Table 21-1): (a) an N-terminal sequence that encodes a serine/threonine kinase, (b) a central portion that encodes a guanidine nucleotide exchange factor, and (c) a C-terminal portion that codes for a guanosine triphosphatase-activating protein (GAP).

### *Properties of* bcr/abl *and Its Protein Product (P210)*

The *bcr/abl* gene encodes a tumor-specific 210-kd hybrid protein (P210) that differs from the normal abl kinase (P145) in several respects: (a) translocation to the cytoplasm, (b) augmented TK activity, (c) ability to autophosphorylate, and (d) ability to bind F-actin. Similar modifications of *c-abl* proteins have been demonstrated to mediate viral oncogenesis (e.g., v-abl and feline sarcoma virus) and to confer growth-factor independence on various cell lines. [19] In each of these instances, the critical genetic alteration involves a substitution at the N-terminal end of the *abl* gene (Fig. 21-3). The N-terminal serine/threonine kinase domain of *bcr* appears to be integral to the activation of abl kinase in the *bcr/abl* fusion molecule; this process involves both a genetic and a physical interaction ( Fig. 21-2).[20,21 and 22] As mentioned previously, under normal conditions, the abl kinase (SH1) is regulated by two other regions of the abl molecule (SH2 and SH3); SH2 exerts a positive regulatory effect on SH1, whereas SH3 is a negative regulator. In the *bcr/abl* fusion molecule (P210), a direct physical binding between the N-terminal sequence of *bcr* and the SH3 regulatory domain of *abl* may deregulate the abl kinase.[22] Two domains within *bcr* are required for its oncogenic effect: domain I mediates oligomerization of *bcr/abl* and promotes phosphorylation of tyrosine residue 177 within domain II, which, in turn, binds to a signaling adapter molecule coupling *bcr/abl* with the *ras* signaling pathway.[23]

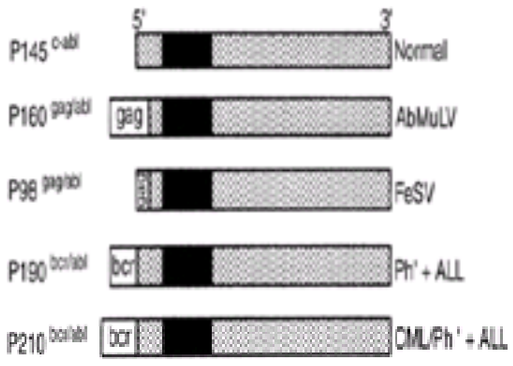


**FIGURE 21-3.** Schematic representation of the normal protein product (P145) of the *c-abl* gene and the modifications associated with leukemogenesis. Note that all variants retain the tyrosine kinase domain (*black area*) and are altered at the 58 end by insertion of the viral gag component in the case of Abelson murine leukemia virus (AbMuLV) and feline sarcoma virus (FeSV) or with bcr segments in the case of Philadelphia chromosome–positive (Ph $^{1}$+) acute lymphocytic leukemia (ALL) and chronic myelocytic leukemia (CML).

As a result of its translocation from the nucleus to the cytoplasm, the constitutively activated *bcr/abl* TK (P210) is exposed to a new spectrum of substrates. Many novel phosphotyrosine-containing proteins are now engendered. Most prominent among these is a 41-kd protein known as *CRKL*.[24,25] CRKL has an overall homology of 60% to CRK (the human homolog of the avian sarcoma viral crk oncogene); like abl protein, CRKL is an adapter protein containing SH2 and SH3 domains that serve as an intermediate in coordinating signal transduction in TK cascades. In normal hematopoietic cells, CRKL is only present in a nonphosphorylated form and, as such, may play a role in differentiation; the constitutive phosphorylation of CRKL in the CML cell may interfere with its ability to complex with other proteins, thereby resulting in loss of function, blocked differentiation, and an expanding pool of immature myeloid precursors. [24,25]

Other phosphorylated proteins found in the CML cell include rasGAP and its associated proteins (p190 and p62), other adapter proteins (GRB-2 and SHC), and the BCR protein itself.[26,27 and 28] These interactions may directly link P210 with the *ras* signal transduction pathway that is central to the regulation of cell proliferation and differentiation.[28] This is consistent with evidence that implicates up-regulation of *ras* function as an essential component of *bcr/abl*'s ability to induce malignant transformation.[29]

### *Other Cytogenetic Consequences of t(9;22)(q34;q11)*

Another gene located on 22q11 and included in the t(9;22) encodes for a cytokine with a broad spectrum of biologic activities, including leukemia inhibitory factor and human interleukin (IL) for DA1 cells. Abnormal regulation of this gene may contribute to the disordered hematopoiesis characteristic of CML. [30]

A reciprocal fusion gene, *abl/bcr*, is formed on chromosome 9q+; this gene, which is also actively transcribed in most patients with CML, has not been well defined. [31] The t(9;22) also results in transposition of c-*sis*, the human homolog of the simian sarcoma virus oncogene, from chromosome 22 to chromosome 9. Normally, c-*sis* encodes sequences of the b-chain of platelet-derived growth factor. Although neither the structure nor the expression of c-*sis* is altered by the translocation event, a subtle transformation of this fibroblast-stimulating gene may possibly play a role in the myelofibrosis seen in some patients with CML.

### *Alternate Splicing Patterns for* bcr/abl

In the genesis of *bcr/abl*, the break in *abl* typically occurs in the first intron between alternate exons la and lb; the break in *bcr* occurs most often between exons 2 and 3 or 3 and 4. During transcription, *abl* exon lb is spliced out, and *abl* exon 2 is spliced to either *bcr* exon 2 or 3. As shown in Fig. 21-4, two distinct messenger RNA (mRNA) species may be encoded (b2a2 or b2a3); these vary in size by 75 bases, and their protein products differ by 25 amino acids. The influence of the splice site on the clinical course of CML has been the subject of much discussion, but no clear conclusion is yet available. [32,33 and 34]

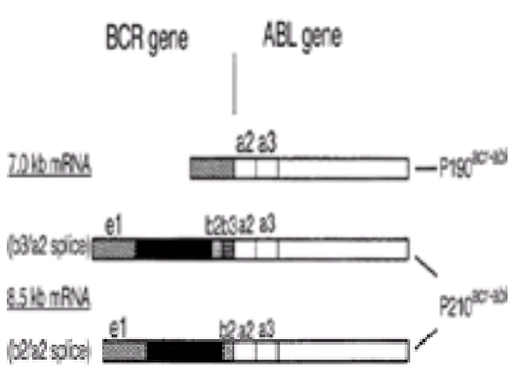


**FIGURE 21-4.** A schematic diagram of alternate splicing patterns for hybrid *bcr/abl* messenger (mRNA). (See text for explanation.) [From Mills KI, Benn P, Birnie GD. Does the breakpoint within the major breakpoint cluster region (M-*bcr*) influence the duration of the chronic phase in chronic myeloid leukemia? An analytical comparison of current literature. Blood 1991;78:1155, with permission.]

As discussed in the section Philadelphia Chromosome–Positive Acute Leukemia, clinicians also recognize an entity known as *Ph$^{1}$-positive acute leukemia.* Approximately half the adults with this condition have *bcr/abl* rearrangements similar to those seen in CML. In the remainder and in nearly 80% of children with Ph$^{1}$-positive acute leukemia, a different rearrangement occurs, with *abl* exon 2 spliced to an exon outside the major *bcr* region (known as *the minor bcr region*).[33] The resultant protein product (P190) has a molecular weight of 190 kd and has approximately a fivefold higher level of TK activity than does P210. This characteristic appears to correlate with a higher transforming ability of P190. Careful analysis has shown that the p190 $^{bcr/abl}$ transcript, traditionally associated with Ph$^{1}$-positive acute leukemia, can be detected at very low levels (corresponding to approximately 0.02% of the total *bcr/abl* transcripts) at diagnosis in virtually all CML patients, apparently arising as a consequence of alternative or missplicing events in the BCR gene. [35] As discussed in the section Detection of Residual Leukemia after Bone Marrow Transplant, quantitation of p190$^{bcr/abl}$ transcript can be utilized to assess total tumor burden and as an early indicator of molecular relapse. [36]

A third *bcr/abl* fusion protein ($p230^{bcr/abl}$) has been associated with a relatively indolent form of chronic leukemia known as *chronic neutrophilic leukemia*. Because the various *bcr-abl* oncoproteins share the same *abl* TK sequences, their different clinical phenotypes must reflect modulation by the unique protein domains contributed by the various *bcr* breakpoints (Table 21-2).[37]

| Fusion gene | *bcr* domains | Clinical phenotype |
|---|---|---|
| P185 (or P190) | Dimerization<br>Binding<br>SH2<br>Serine/threonine kinase | $Ph^1$+ acute leukemia |
| P210 | All of the above plus:<br>Dbl-like<br>Pleckstrin homology | $Ph^1$+ chronic myelocytic leukemia |
| P230 | All of the above plus:<br>Calcium-phospholipid binding<br>One-third of GTPase activating gene for p21 rac (rac-GAP) | Chronic neutrophilic leukemia |

GAP, guanosine triphosphate-activating protein; GTP, guanosine triphosphate.

**TABLE 21-2. RELATIONSHIP OF BCR DOMAINS TO *BCR/ABL* FUSION GENES**

Although the classic t(9;22) is found in approximately 90% of patients with CML, in some instances, other types of translocations may be found.[38,39,40,41] and [42] Approximately 3% of patients with CML have translocations of 22q11 to regions other than 9q34. Another 3% have complex translocations involving three or more chromosomes; such translocations virtually always involve band 9q34. Other patients may have an undetected or "masked" $Ph^1$ chromosome (see $Ph^1$-negative CML). A few patients have, in addition to the $Ph^1$ chromosome, other visible karyotypic abnormalities such as a second $Ph^1$ chromosome, isochromosome 17, or an extra chromosome 8 or 18; these secondary changes appear to represent a mechanism of tumor progression and are found with increased frequency as the disease evolves to a more aggressive phase.

### *Philadelphia Chromosome–Negative Chronic Myelocytic Leukemia*

Approximately 5% to 10% of patients with otherwise typical CML do not manifest the $Ph^1$ chromosome. In some of these patients, the $Ph^1$ chromosome may be masked by translocation of additional genetic material to the 22q11 region.[41] Other patients may have rearrangements or breaks in the 9q34 region without the reciprocal break at 22q11.[17,42] Molecular biology techniques permit identification of *bcr* rearrangements in $Ph^1$-negative patients who have otherwise typical CML.[43,44] In such cases, *abl* and *bcr* are presumed to have been juxtaposed on the molecular level by a mechanism other than that producing the typical t(9;22); this phenomenon may result from an interstitial insertion of *abl* into *bcr* or a complex translocation with the *bcr/abl* fusion gene located on another chromosome.

Patients with a CML-like picture but lacking both $Ph^1$ and *bcr* rearrangement have also been described; such patients appear to have a distinct clinical course characterized by increasing leukocytosis, organomegaly, extramedullary infiltrates, and eventual bone marrow failure.[45]

### *Philadelphia Chromosome–Positive Acute Leukemia*

Although characteristic of CML, the $Ph^1$ chromosome is not exclusive to it. This chromosomal abnormality is found in approximately 3% to 10% of childhood acute leukemias, in 2% to 3% of adult acute myeloid leukemias, and in 25% to 33% of adult acute lymphoid leukemias.[46,47] and [48] $Ph^1$-positive acute leukemias have no antecedent CML features and are clinically and hematologically indistinguishable from other acute leukemias except for a relatively poorer prognosis. In a review of 267 patients with $Ph^1$-positive acute lymphocytic leukemia (ALL), Arico and colleagues[49] found that complete remission was achieved in 82% of cases. These patients could be subsequently stratified into three prognostic groups based on age and initial white blood cell count (WBC) ( Table 21-3). Intensive chemotherapy gave modestly successful results in the most favorable group; however, bone marrow transplant from an HLA-matched donor appears to be the preferred modality for most $Ph^1$-positive ALL patients.

| Prognostic group | Age | Initial white blood cell count | 5-yr disease-free survival |
|---|---|---|---|
| Favorable | <10 | <50,000/mm$^3$ | 49 ± 5% |
| Intermediate | >10 and/or | 50,000–100,000/mm$^3$ | 30 ± 5% |
| Poor prognosis | Any | >100,000/mm$^3$ | 20 ± 5% |

**TABLE 21-3. PROGNOSTIC CATEGORIZATION OF PHILADELPHIA CHROMOSOME–POSITIVE ACUTE LYMPHOCYTIC LEUKEMIA**

Some $Ph^1$-positive acute leukemias may represent blastic presentations of CML, whereas others are apparently true de novo acute leukemias. Few clinical or hematologic features enable the clinician to distinguish between these two entities, although cases associated with basophilia or marked splenomegaly are more likely to be associated with CML. To confuse the issue further, classic chronic-phase CML developed after a period of hematologic and cytogenetic remission in a patient who initially presented with apparent de novo $Ph^1$-positive acute leukemia.[50]

Differences between blastic-phase CML and de novo $Ph^1$-positive acute leukemia are more apparent at the cytogenetic and molecular levels. $Ph^1$-positive acute leukemias usually do not manifest the specific nonrandom chromosomal aberrations (discussed in the section Cytologic Heterogeneity of Blast Phase) that are characteristic of CML as it evolves into blastic phase. Furthermore, the marrow karyotype of the de novo acute $Ph^1$-positive leukemia case usually reverts to normal after therapy, whereas the $Ph^1$ chromosome persists in the bone marrow of the patient with CML. Patients with CML, regardless of phase, almost universally exhibit typical *bcr* rearrangements and production of P210. On the other hand, approximately one-half the patients with de novo $Ph^1$-positive acute leukemias (and virtually all children with this condition) show rearrangements outside the *bcr* region and produce the 190-kd protein.[51,52]

## Biology

The cytogenetic changes in the CML precursor cell are expressed in its descendants by a variety of cytologic alterations that, in turn, produce the neoplastic phenotype (Table 21-4). These biologic features are discussed in the subsequent sections.

| Abnormality | Consequence |
|---|---|
| Reduced adherence to stromal matrix | Decreased stroma/stem cell interaction<br>Abrogation of normal cell surface signal maturation |
| Discordant nuclear:cytoplasmic maturation | Prolongation of late progenitor proliferative phase<br>Opportunity for further divisions before maturation |
| Failure of apoptosis program | Prolonged survival and increased accumulation |
| Production of inhibitory molecules | Suppression of normal hematopoietic stem cells |
| Insensitivity to regulatory molecules | Selective growth advantage for CML cells |

**TABLE 21-4. CYTOLOGIC ABNORMALITIES IN CHRONIC MYELOCYTIC LEUKEMIA (CML)**

### *Clonality*

Independent lines of evidence derived from cytogenetic data and analysis of isoenzyme patterns indicate that CML is an acquired disorder of unicellular origin, and the target of neoplastic transformation is a multilineage stem cell with the potential for generating all the hemic cells (i.e., erythrocytes, neutrophils, basophils, eosinophils, monocytes, and megakaryocytes) and, in at least some instances, the lymphoid lineages. This multilineage potential accounts for the cytologic heterogeneity of the blastic phase of CML (discussed later under Natural History).

#### Cytogenetic Data

The $Ph^1$ chromosome has proved useful in defining the malignant population in CML. This acquired abnormality is demonstrable in virtually all proliferating erythroid, granulocytic, monocytic, and megakaryocytic precursor cells but not in fibroblasts or other somatic cells. A similar pattern of clonal restriction can be demonstrated for other karyotypic markers, such as that found in CML patients with coincident sexual mosaicism or Down syndrome.[53] Routine karyotypic studies do not indicate the presence of the $Ph^1$ chromosome in the lymphocytes of patients with CML. The phenomenon of lymphoblastic transformation in some patients with CML, however, suggests that at least some lymphoid lineages are involved in the malignant process. This suggestion is supported by demonstration of the $Ph^1$ chromosome in a small proportion of B lymphocytes and T lymphocytes when CML blood is cultured *in vitro*.[54] Other studies have documented the presence of $Ph^1$-positive B and T lymphocytes in multilineage colonies derived from CML precursor cells[55]; furthermore, *bcr/abl* transcripts have been detected in T lymphocytes of patients with CML.[56] In comparison with hemic cells, lymphoid cells are relatively long-lived, and therefore most of those present at diagnosis of CML probably antedate the neoplastic transformation event; this may explain the relatively small fraction in the malignant clone.

#### Isoenzyme Patterns

In accordance with the Lyon hypothesis, random inactivation of one X chromosome occurs in each cell during early embryogenesis; the progeny of each of these cells subsequently manifests the same pattern of X chromosome inactivation in a clonal fashion. Because approximately one-half the cells express one X chromosome and half the other X chromosome, females who are heterozygous for an X-linked enzyme should have roughly equal proportions of the two isoenzymes in each tissue. On the other hand, a neoplastic clone arising from a single cell should manifest only a single isoenzyme pattern. Studies of females with CML who are heterozygous for the X-linked enzyme glucose-6-phosphate dehydrogenase have demonstrated that the normal somatic tissues show the expected double isoenzyme distribution, whereas the $Ph^1$-positive hemic lineages contain a single (clonal) isoenzyme phenotype.[57,58] The demonstration that $Ph^1$-positive B lymphocytes also manifest a clonal pattern of glucose-6-phosphate dehydrogenase distribution confirms that this lineage is also derived from the CML stem cell[59,60]; this finding has important implications with regard to the initial transformation event in CML.

### *Initial Transformation Event*

Although clearly germane to the pathogenesis of CML, the formation of the $Ph^1$ chromosome may not be the primary event in the neoplastic sequence. In some documented cases, the typical clinical and hematologic abnormalities of CML antedated the appearance of the $Ph^1$ chromosome[61,62]; in other cases, the $Ph^1$ chromosome disappeared during therapy while the clinical and hematologic features of CML persisted.[63] Isoenzyme studies that indicate clonality of some Ph-negative lymphoid populations also imply a transformation event preceding the (9;22) translocation.[60] Investigators have suggested that a rearrangement or break in the 9q34 band (the locus of *c-abl*) may be the critical initiating step in the evolution from benign to malignant hematopoiesis.[17]

### *Mechanisms Underlying Growth Advantage of Chronic Myelocytic Leukemia Cells*

The expansion of the CML clone from a single transformed cell to predominance in bone marrow and blood is dramatic evidence of its ability to overgrow the normal hemic elements. The mechanisms by which it achieves preeminence have been of great interest to students of the disease. Some clues have been provided by analysis of proliferative kinetics and *in vitro* colony production.

#### Cell Kinetics

In chronic-phase CML, one sees a threefold to 30-fold increase in peripheral blood band and segmented neutrophils and a tenfold to 100-fold increase in the total blood granulocyte pool (TBGP).[64,65,66 and 67] These leukocytes freely exchange between the peripheral blood, bone marrow, and spleen. The average one-half-life of granulocytes in the blood is five to ten times longer in patients with CML than in healthy persons; this phenomenon is partially a reflection of the large number of immature forms, but even the morphologically mature granulocytes of CML have a peripheral blood half-life twofold to fourfold longer than normal.[66] This increased blood transit time contributes to the expansion of the TBGP. Of greater significance, however, is the increased granulocyte production rate.

The hyperproduction of myeloid cells in CML is not attributable to unregulated or unduly rapid proliferation; indeed, measurements of mitotic activity, DNA synthesis, and generation time indicate that CML progenitor cells divide more slowly than normal hemic precursor cells.[64,65,66 and 67] An overproduction of committed myeloid precursor cells can be demonstrated, however, by assays of the colony-forming unit–granulocyte-macrophage (CFU-GM).[67,68,69 and 70] Bone marrow suspensions from patients in chronic-phase CML generate ten to 20 times the number of GM colonies produced by normal marrow. The disparity is even more striking when peripheral blood is studied. The majority of circulating CFU-GM may be generated in the spleen.[71] Colony growth is qualitatively normal (in chronic phase); mature GM forms are produced, and the process depends on the same colony-stimulating factors (CSFs) that are obligatory for *in vitro* colony formation by normal CFU-GM. After chemotherapy or splenic irradiation, the TBGP and the half-life of circulating granulocytes return to normal values coincident with a reduction in the marrow cellularity, a reduction of the bone marrow and peripheral blood CFU-GM, and disappearance of immature cells from the peripheral blood. This change suggests that most of the kinetic abnormalities found in chronic-phase CML do not reflect an intrinsic maturational defect of the neoplastic cells, but are a consequence of the premature release of immature cells from the marrow resulting from the mechanical pressure of an increased granulocytic cell mass. The basic defect appears to be discordant maturation with preferential expansion of the progenitor compartment.[67]

As the disease progresses to blast phase, defects in maturation become more conspicuous. Myeloblasts become abundant in the bone marrow and peripheral blood, whereas the relative and absolute numbers of polymorphs decline. An inverse correlation exists between the percentage of myeloblasts in the marrow and the fraction of these cells in DNA synthesis.[72] This decline in proliferative and maturational potential is reflected in absent or reduced GM colony production *in vitro*.

#### Abnormalities in Feedback Regulation

Under normal circumstances, myelopoiesis is regulated by at least three negative feedback molecular species: lactoferrin (LF), prostaglandin E (PGE), and acidic isoferritins (AIFs) (Fig. 21-5). LF, the product of mature polymorphonuclear leukocytes (PMNs), down-regulates granulocytopoiesis by reducing monocyte-macrophage production of CSF.[73] PGE and AIF, which are derived from subpopulations of monocytes and macrophages, inhibit proliferation of normal

granulocyte-monocyte precursor cells;[73,74] their inhibitory actions appear to be restricted to those cells that express HLA-DR (Ia) antigens.[75,76]

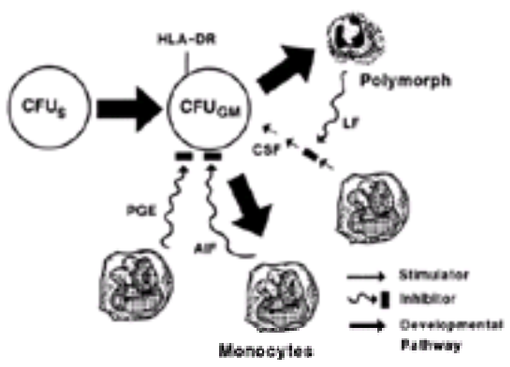


**FIGURE 21-5.** Positive and negative feedback loops regulating myelopoiesis. In this postulated schema, progeny of the pluripotent stem cells (CFU-S) become committed granulocyte-monocyte precursor cells (CFU-GMs). CFU-GMs are induced to proliferate and produce polymorphs and monocytes by colony-stimulating factors (CSFs). CFU-GMs are inhibited from proliferating by lactoferrin (LF) (which inhibits generation of CSF) and by acidic isoferritins (AIFs) and prostaglandin E (PGE) (which act directly on CFU-GMs bearing HLA-DR antigens). In chronic myelocytic leukemia, the neoplastic clone may derive its growth advantage by modifications of these regulatory pathways.

CML cells appear to be relatively insensitive to feedback inhibition by virtue of deficient LF production by the PMNs, decreased responsiveness of the monocyte-macrophage to regulation by LF, and decreased sensitivity of progenitor cells to PGE and AIF.[77,78] The PGE and AIF resistance may reflect deficient HLA-DR antigen expression by CML cells or a decreased proportion of sensitive target cells. CML cells may augment their proliferative advantage by releasing humoral factors to which they are resistant, but that suppress normal hemopoietic precursor cells.[79]

### Altered Adhesive Interactions

Under normal conditions, hematopoietic progenitor cells adhere to the extracellular matrix protein fibronectin in bone marrow stroma via fibronectin receptors (integrins). $Ph^1$+ progenitor cells exhibit reduced adhesion to fibronectin. This may result from an acquired functional defect of the fibronectin receptor or its downstream signaling pathways or both. Several cytoskeletal proteins associated with integrin regulation (e.g., paxillin, FAK, CKRL, and vinculin) are phosphorylated by the *bcr/abl* TK, and their altered status may contribute to abnormal integrin function.[80] Deficient binding of CML progenitors to marrow stroma may lead to release of immature cells into the circulation and may facilitate hematopoiesis in extramedullary sites. Conversely, this defect in adhesiveness may facilitate selection for normal ($Ph^1$-negative) stem cells *in vitro* by inhibiting the survival of CML progenitor cells in long-term culture systems.

### Resistance to Apoptosis

The expansion of a malignant clone reflects an imbalance between the rate of cell proliferation and the rate of cell death. As discussed in the section Cell Kinetics, studies of the kinetics of CML cells suggest that they are not produced at an increased rate; therefore, the massive accumulation of cells in CML probably results from prolongation of cell survival. Under normal circumstances, hemic cells have a limited life span that is regulated by a genetic program of active autonomous cell death (apoptosis); the presence of the *bcr/abl* fusion protein appears to render CML cells resistant to the induction of apoptosis.[81,82] The *bcr/abl* oncoproteins protect cells from apoptosis via several different mechanisms, among which are: inhibition of upstream preapoptotic mitochondrial events (e.g., release of cytochrome C) and downstream inhibition of caspase 3.[83] Many of these effects are mediated by influencing the relative expression levels of apoptosis inhibitors (e.g., BCL-2, BCL-$X_L$) and promotors (e.g., BAX, BAD, BCL-$X_S$) or the subcellular localization of these factors, or both.

In addition to promoting leukocytosis, suppression of apoptosis may also allow cells to accumulate new genetic changes with consequent neoplastic progression. The *bcr/abl*-mediated inhibition of apoptosis is also associated with prolongation of cell cycle arrest at the $G_2$/M restriction point; this delay may give the cell time to repair chemotherapy-induced damage to DNA rather than proceeding to programmed cell death.[84] Because response to many chemotherapeutic agents depends on activation of the apoptosis program, inhibition of apoptosis may limit the susceptibility of CML cells to standard cytotoxic chemotherapy. Thus, resistance to apoptosis may be implicated in several important features of CML: massive clonal expansion, neoplastic progression, and resistance to chemotherapy.

### *Acute Transformation*

In the course of CML, progressively more abnormal stem cell clones evolve sequentially from the original $Ph^1$-positive clone. With the development of new cytogenetic alterations is an increasing dissociation between proliferation and differentiation. The newly evolved clones suppress proliferation of both normal stem cells and the cells of antecedent leukemic clones. Eventually, immature (blast) cells predominate, and the process terminates as an acute leukemia.

In the Preisler model of leukemogenesis,[85] the process of acute transformation involves at least two complementary gene families (Table 21-5). Class I genes code for signal transduction proteins that participate in the chain of reactions that transmit signals from the cell surface to the cytoskeletal elements or the nucleus; oncogenic transformation usually produces an abnormal protein that induces abnormalities of proliferation or differentiation. Class II genes code for proteins that interact directly with the genome and regulate the expression of other families of genes; oncogenic transformation usually results in an abnormality in regulation of expression leading to increased proliferative potential or immortalization.

| Class I (signal transducers) | | Class II (signal effectors) | |
|---|---|---|---|
| **Gene** | **Locus of action** | **Gene** | **Locus of action** |
| *sis* | External milieu (PDGF) | *myc* | Nucleus |
| *erb-b* | Cell membrane (EGF-R) | *p53* | Nucleus |
| *fms* | Cell membrane (CSF-1-R) | *rb* | Nucleus |
| *ras* | Submembrane (G protein) | | |
| *abl* | Submembrane (tyrosine kinase) | | |
| *src* | Submembrane (tyrosine kinase) | | |
| *mos* | Cytoplasm | | |
| *raf* | Cytoplasm | | |
| *mil* | Cytoplasm | | |

EGF-R, epidermal growth factor receptor; CSF-1-R, colony-stimulating factor-1 receptor; PDGF, platelet-derived growth factor.

**TABLE 21-5. CLASSIFICATION OF PROTO-ONCOGENES**

Applying the Preisler model to CML, the chronic phase may be attributed to an abnormal protein (P210) derived from an altered class I protooncogene (*c-abl*). Progression to acute phase does not appear to result from a further change in the *bcr/abl* gene, nor are alterations in other class I genes expected to induce acute transformation. Instead, alteration of a class II gene is more likely to complement the oncogenic potential of the preexisting class I genetic mutation. The low incidence of N-*ras* (class I) mutations and the relatively high incidence (22 to 30%) of p53 (class II) mutations seen in association with blastic transformation of CML are consistent with this model of leukemogenesis.[86,87,88] and [89]

### *Multistep Pathogenesis*

The evolution of CML may be visualized as a multistep process that can be summarized as follows:

1. An initial transformation event (possibly rearrangement of *c-abl*) occurs in a multipotent progenitor cell. This results in production of a clone of "premalignant" hemic (and possibly lymphoid) cells. These cells may be metabolically defective and require a subsequent genetic mutation (e.g., creation of the *bcr/abl* fusion gene) to compensate for the defect and "rescue" them from impending apoptosis.[90]
2. At some point in the evolution of the disease, a recognizable cytogenetic alteration (the Ph [1] chromosome) appears in the transformed clone. The translocation of *c-abl* to chromosome 22 and its fusion with the *bcr* unmask or deregulate TK activity, which, in turn, produces novel tyrosine phosphorylated proteins (e.g., CKRL). The $Ph^1$-positive clone now acquires a growth advantage over normal hemic stem cells, and initially an overproduction of relatively mature cells occurs, particularly those of the granulocytic series.
3. Genomic instability and spontaneous errors in DNA replication may promote the appearance of progressively more abnormal stem cell clones derived from the original $Ph^1$-positive clone; new cytogenetic alterations appear, and an increasing dissociation exists between proliferation and differentiation. The newly evolved clones suppress the proliferation of normal stem cells, as well as the cells of the preceding leukemic clones. Eventually, immature (blast) cells predominate, and the process terminates in an acute leukemia.

## Natural History

The natural history of CML is divided into chronic, accelerated, and blast phases. These phases represent the progressive shift in the nature of the disorder from one of hyperproliferation, with production of mainly mature hemic elements, to one characterized by a differentiation arrest, with production of predominantly immature (blast) cells.

### *Chronic Phase*

The chronic phase is characterized by marked expansion of the hematopoietic pools; morphologically mature blood cells are produced that show only subtle functional abnormalities. Generally, the neoplastic cells are restricted to the bone marrow, liver, spleen, and peripheral blood. Therefore, symptoms are related to organ infiltration, hyperviscosity, and the metabolic consequences of hyperproliferation, all of which are relatively easy to control. On average, the chronic phase lasts approximately 3 years.

## Symptoms

Patients usually present with nonspecific complaints, such as fever, night sweats, weakness, left upper quadrant pain or fullness, and bone pain. Neurologic dysfunction, respiratory distress, visual difficulties, or priapism may complicate cases characterized by marked hyperleukocytosis.

## Physical Findings

The usual physical findings in this phase of CML are pallor, low-grade fever, ecchymoses, hepatosplenomegaly, and sternal tenderness. Signs relating to leukostasis (e.g., neurologic abnormalities, papilledema, retinal hemorrhages, and tachypnea) are seen in patients with extreme hyperleukocytosis.

## Laboratory Findings

A mild normochromic, normocytic anemia, marked leukocytosis with "shift to the left," and thrombocytosis are common laboratory findings. The mean hematocrit at presentation in children (25 mL per dL) is significantly less than that seen in adults.[12,91] The leukocyte count at diagnosis ranges from approximately 8,000 to 800,000 per $mm^3$; the median count in children (approximately 250,000 per $mm^3$) is higher than that seen in adults.[11,12] Extreme hyperleukocytosis (greater than 500,000 per $mm^3$) is also more common in children. The peripheral blood smear shows myeloid cells at all stages of differentiation; myeloblasts and promyelocytes generally comprise less than 15% of the differential count, and no hiatus leukemicus in maturation occurs ( Fig. 1-6). An absolute increase in the numbers of basophils and eosinophils is noted. Hybrid eosinophilic-basophilic granulocytes may also be seen [92]; because similar chimeric granules may be found in normal immature granulocytes, this phenomenon may reflect incomplete maturation.[67] The mean platelet count in children is approximately 500,000 per $mm^3$, which is not significantly higher than that in adults.[91] Serologic findings include elevation of uric acid, lactate dehydrogenase, vitamin $B_{12}$, and vitamin $B_{12}$–binding protein (transcobalamin 1).

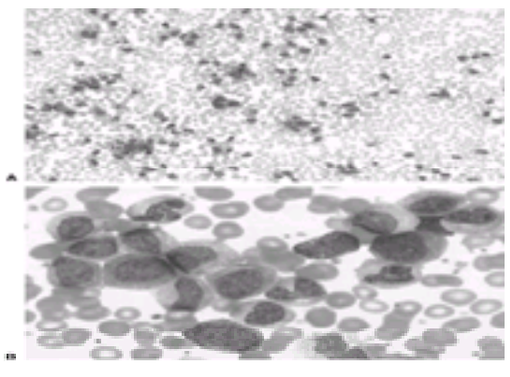

**FIGURE 21-6.** Peripheral blood smear of chronic-phase chronic myelocytic leukemia. **A:** Low-power magnification showing marked leukocytosis. **B:** High-power magnification showing the entire range of myeloid cells from myeloblast to mature polymorphonuclear leukocytes. (Courtesy of Dr. William Rezuke.) (See Color Figure 21-6.)

The bone marrow is hypercellular, mainly reflecting granulocytic (and often megakaryocytic) hyperplasia; orderly granulocyte maturation, eosinophilia, and basophilia are present. Myelofibrosis, which occurs in 30% to 40% of patients during the course of the disease, is uncommon in the early chronic phase. The bone marrow and spleen occasionally contain lipid-laden histiocytes that resemble Gaucher cells or sea-blue histiocytes.

The characteristic histochemical abnormality of the granulocyte population is reduction in leukocyte alkaline phosphatase (LAP) activity. Although this abnormality does not appear to affect PMN function adversely, it is of diagnostic utility in distinguishing the leukocytosis of CML from that of inflammation (in which LAP is generally elevated). Low LAP activity is also seen in paroxysmal nocturnal hemoglobinuria, JMML, CMML, and Fanconi's anemia. Under normal conditions, LAP activity appears very late in the development of granulocytes, and, indeed, may be a terminal marker of granulocyte maturation. [93] In CML, there appears to be no intrinsic defect in the synthesis and translation of LAP mRNA or transport of LAP protein to the plasma membrane; furthermore, LAP activity can be up-regulated under a variety of conditions [e.g., inflammation, leukoreduction, disease progression, and administration of granulocyte colony-stimulating factor (G-CSF)]. Thus, the low LAP levels in CML cells may result from granulocyte immaturity or hypoproduction of G-CSF due to a relative decrease in monocyte mass.[93,94]

LAP activity increases with infection or with a reduction of the granulocyte count after chemotherapy or progression to a more acute phase of the disease. Although subtle functional abnormalities of PMN adherence, chemotaxis, bactericidal activity, and membrane sialylation can be demonstrated in chronic-phase CML, the PMNs are sufficiently effective to prevent infectious complications.[95,96] PMN function deteriorates progressively as the disease evolves.

## Differential Diagnosis

The differential diagnosis of chronic-phase CML includes leukemoid reaction, JMML, and other myeloproliferative disorders. The combination of low LAP score and

the presence of the Ph[1] chromosome usually distinguishes CML from these conditions.

In leukemoid reactions, splenomegaly is usually not marked, the LAP score is high, the Ph [1] chromosome is absent, and an inflammatory focus is often demonstrable. In JMML, the LAP may be low, but the Ph[1] chromosome is absent; leukocytosis and splenomegaly are less marked than in CML, and involvement of skin, lymphoid tissue, and the monocytic lineage is more pronounced. CML can be distinguished from other myeloproliferative disorders by the disproportionate involvement of the granulocyte series and the presence of the Ph[1] chromosome.

***Metamorphosis***

After approximately 3 years, the chronic phase of CML undergoes a metamorphosis into a more aggressive phase; this may occur gradually or abruptly. In approximately 5% of cases, the evolution is explosive, with a rapidly increasing blast cell population in the peripheral blood and concurrent neutropenia and thrombocytopenia ("blast crisis"). Approximately 50% of patients develop a progressive maturation defect resulting in a hematologic picture similar to that of de novo acute leukemia; the remaining 45% have the gradual evolution of a myeloproliferative syndrome.

The onset of metamorphosis is characterized by progressive systemic symptoms (e.g., fever, night sweats, and weight loss), increasing leukocyte counts with a high proportion of immature cells, basophilia, and increasing resistance to chemotherapy. Along with these features is evidence of karyotypic evolution. Mutations in the antioncogene p53 may play a significant role in transformation: p53 mutations are detectable in the late chronic phase of CML and may indicate increasing genomic instability and early progression to blast transformation. [97,98] New karyotypic abnormalities (most commonly, duplication of the Ph[1] chromosome, isochromosome 17, or trisomy 8) also begin to appear. Occasionally, the first manifestation of metamorphosis is extramedullary (i.e., meningeal leukemia or a chloroma arising in soft tissue or bone); such findings usually herald the imminent blast transformation of the marrow.

***Blast Phase***

The blast phase is characterized by loss of the leukemic clone's capacity to differentiate. As a consequence, the clinical picture resembles that of an acute leukemia, with anemia, thrombocytopenia, and increased numbers of blast cells in both the peripheral blood and the bone marrow. A marrow blast percentage of 30% or more is diagnostic of blast phase. The signs and symptoms are those of a de novo acute leukemia; if basophilia is extreme, the patient may also have hyperhistaminemic symptoms (e.g., pruritus, cold urticaria, and gastric ulceration). When the absolute blast count exceeds 100,000 per mm [3], the patient is at risk for hyperleukocytosis syndrome with leukostasis.

**Cytologic Heterogeneity of Blast Phase**

As a reflection of the pluripotent nature of the leukemic stem cells in CML, blast transformation may involve any of the lymphohematopoietic lineages. In approximately 60% to 70% of cases, the blast cell morphology is myeloblastic; unlike de novo acute myelocytic leukemia, however, the blast cells are usually peroxidase negative and rarely have Auer rods. Careful analysis using lineage-specific markers, such as glycophorin-A, platelet peroxidase, and monoclonal antibodies, enables clinicians to identify some of these blast transformations as erythroid, monocytic, or megakaryocytic. [99] Approximately one-third of patients have blast cells with lymphoid morphology. These cells generally express a phenotype corresponding to an early B cell. [99,100] In rare cases, blast cells may express T-lineage markers; these patients usually have marked extramedullary involvement (especially in lymph nodes) and frequently lack a preceding chronic phase. [101] In some patients, the blast cells manifest features of more than one myeloid line or have mixed myeloid-lymphoid features.

The course of transformation of CML from chronic phase to the acute (blastic) phase is often accompanied by further cytogenetic changes. In transformation, the most commonly identifiable karyotypic alterations are duplication of the Ph [1] chromosome (+Ph[1]), trisomy 8 (+8), trisomy 19 (+19), and isochromosome 17q (i17q). A rare consistent chromosomal abnormality, a reciprocal t(3;21)(q26;q22), has been described in patients with CML either before or at the onset of blast transformation. [102,103] and [104] In general, these secondary cytogenetic changes are nonspecific and may indicate a generalized genomic instability rather than directly relate to blastic progression[101]; however, other changes clearly mirror the karyotypic features associated with specific subtypes of de novo acute leukemia and may be directly related to blastic transformation (Table 21-6).[105] The phenotypic features of blast transformation may be determined by these specific secondary genetic events; for example, rearrangements involving chromosome regions containing immunoglobulin genes and T-cell receptor genes have been associated with B-lymphoid and T-lymphoid acute transformations, whereas involvement of region 3q21 may be accompanied by dysmegakaryopoiesis and conversion to acute megakaryoblastic leukemia.[98,103,104]

| | Acute phase | |
|---|---|---|
| | Myeloid | Lymphoid |
| Incidence of abnormalities | 80% | 30% |
| Ploidy | Hyper | Hypo, Pseudo |
| Nonrandom chromosome abnormalities | | |
| +Ph | + | + |
| +21 | + | + |
| aY | + | + |
| i(17q) | + | - |
| +8 | + | +- |
| +19 | + | - |
| t(7;11) | + | +- |
| Specific rearrangements: | t(15;17)(q22;q?12)[a] | inv(7)(p15q34)[c] |
| | 3q21-3q26(inv. or t)[b] | t(14,14)(q11;q32)[d] |

[a]Seen in association with acute promyelocytic leukemia.
[b]Seen in association with acute megakaryoblastic leukemia.
[c]Seen in association with acute T-cell lymphoblastic leukemia.
[d]Seen in association with acute B-cell lymphoblastic leukemia.
Modified from Bernstein R, Gale RP. Do chromosome abnormalities determine the type of acute leukemia that develops in CML? Leukemia 1988;2:65.

**TABLE 21-6. ASSOCIATION BETWEEN CHROMOSOME ABNORMALITIES AND ACUTE PHASE CHRONIC MYELOCYTIC LEUKEMIA**

**Differential Diagnosis**

Because most instances of blast-phase CML occur after a well-documented chronic phase, the diagnosis is usually clear-cut. The rare patient who presents in blast phase without a recognized preceding chronic phase may pose diagnostic difficulty, however. The combination of marked splenomegaly, basophilia, and the Ph [1] chromosome distinguishes blast-phase CML from most types of de novo acute leukemia; the distinction between blast-phase CML and de novo Ph [1]-positive acute leukemia is discussed in the section Philadelphia Chromosome–Positive Acute Leukemia.

**Prognostic Considerations**

Recent advances in the management of CML have improved median survival to 5.0 to 5.5 years (previously 3 to 4 years); 35% to 40% of patients survive 7 to 8 years. Because patients generally die within months of transformation to accelerated or blast phase, the major determinant of survival is the duration of the chronic phase, which can be highly variable. For adults, factors at diagnosis that predict early transformation include splenomegaly (>15 cm below costal margin), hepatomegaly (>6 cm below costal margin), thrombocytopenia (less than 150,000 per mm[3]), thrombocytosis (greater than 500,000 per mm[3]), marked leukocytosis (greater than 100,000 per mm[3]), and high proportions of blast cells or immature granulocytes (greater than 1% or greater than 20%, respectively). [106,107] The role of these factors in the prognosis of pediatric patients with CML is less clear; in one study, only peripheral blood and marrow blast counts at presentation were of prognostic significance. [11]

Once patients have entered the blast phase, the only parameters that correlate with survival are blast cell phenotype and cytogenetic findings. In general, lymphoblastic phenotype and minimal karyotypic evolution augur a more favorable response to therapy. [108]

**Therapy**

Achievement of true remission in CML would require destroying all Ph [1]-positive cells and replacing them with cytogenetically normal precursors. This goal is rarely accomplished with conventional therapeutic approaches, but bone marrow transplantation (BMT) has achieved apparent cure in a significant minority of patients. The

initial goal of treatment for patients in chronic phase has been to provide symptomatic relief by ameliorating leukocytosis and organomegaly. For patients in accelerated or blast phase, the goal is reversion to chronic phase. Before initiating any specific antileukemic therapy, consideration must be given to metabolic, leukostatic, and meningeal complications that pose management problems.

### *Special Management Problems*

#### Metabolic Disorders

Metabolic consequences of rapid cytolysis (e.g., hyperuricemia, hyperkalemia, and hyperphosphatemia) should be anticipated and treated appropriately with hydration, alkalinization, and allopurinol. [109] A detailed discussion of metabolic management is found in Chapter 38.

#### Hyperleukocytosis

The extremely high leukocyte count associated with some cases of CML can cause leukostatic complications in several organs, especially brain, lung, retina, and penis.[110,111] Because leukocytes are less deformable than erythrocytes, the viscosity of the blood increases dramatically as the fractional volume of leukocytes (leukocrit) increases. Myeloblasts, which are larger and more rigid than other leukocytes, contribute disproportionately to viscosity; thus, the patient with myeloblastic transformation is at particularly high risk. If hyperleukocytosis is symptomatic or extreme (leukocytes greater than 200,000 per $mm^3$ or blast count greater than 50,000 per $mm^3$), it should be treated with the simultaneous use of cytotoxic drugs (e.g., hydroxyurea, 50 to 75 mg per kg per day by intravenous infusion) and leukapheresis (or exchange transfusion); erythrocyte transfusions (which increase blood viscosity) should be avoided if possible until the leukocyte count is reduced to a safe level.

#### Thrombocytosis

Thrombocytosis may be associated with thromboembolic or hemorrhagic complications. If thrombocytosis does not respond to the CML treatment regimen, the use of anagrelide (an agent that prevents megakaryocyte maturation) or thiotepa (75 mg per $m^2$ intravenously every 2 to 3 weeks until response occurs) should be considered.[112]

#### Priapism

Persistent painful penile erection may result from sludging and mechanical obstruction by leukemic cells, coagulation within the corpora cavernosa secondary to thrombocytosis, or impingement by the spleen on abdominal veins and nerves. Treatment includes analgesia, hydration, application of warm compresses, radiotherapy (to penis or spleen), and initiation of high-dose chemotherapy (e.g., hydroxyurea, 50 to 75 mg per kg per day intravenously). [110,111]

#### Meningeal Leukemia

Meningeal leukemia is almost unknown in the chronic phase of CML and is rare in blast phase. The incidence may increase with improved survival of patients with blast transformation. The usual neurologic signs are cranial nerve palsies and papilledema; the diagnosis is confirmed by demonstrating pleocytosis, with blast cells in the spinal fluid. Intrathecal methotrexate is effective therapy, [113] but most patients eventually die of the hematologic consequences of the blast transformation. The role of prophylactic central nervous system therapy is undefined. [114]

### *Cytoreduction in Chronic Phase*

#### Single-Agent Chemotherapy

Single-agent chemotherapy with busulfan or hydroxyurea has traditionally been the standard approach to the chronic phase of CML. However, these agents are becoming replaced with interferon-a (IFN-a), and, more recently, STI-571. The goal is to achieve symptomatic relief by lowering the WBC count and by reducing liver and spleen size. True remission is rare, however; in most cases, the bone marrow continues to manifest granulocyte hyperplasia and Ph $^1$-positive metaphases even after the blood count has normalized and organomegaly has resolved. Therefore, even though symptomatic relief is achieved, the blast crisis is not delayed, and survival is not prolonged.

#### Hydroxyurea

Hydroxyurea is an inhibitor of ribonucleoside diphosphate reductase (an enzyme essential for DNA synthesis) and is specific for cells in S phase of the cell cycle. Unlike busulfan, it is relatively short-acting; however, its control of the leukocyte count is less reliable, and it must be used daily for prolonged periods. The recommended beginning dose is 10 to 20 mg per kg per day, [115] and dosages must be adjusted according to the hematologic response. Hydroxyurea appears to be as effective as busulfan in controlling chronic-phase CML, [116,117] while providing a greater margin of hematologic safety and less systemic toxicity.

#### Busulfan

Busulfan, an alkylating agent, is cell-cycle–phase nonspecific; it is characterized by delayed onset and prolonged duration of effect. The usual dosage is 0.06 to 0.10 mg per kg per day orally (adult dose, 4 mg per day). Once treatment is initiated, a lag of 10 to 14 days occurs before the leukocyte count falls significantly and the differential count begins to normalize. Splenic regression lags behind the peripheral blood response, and it may be 3 months before the spleen is no longer palpable. The dosage should be reduced by 50% when the leukocyte count reaches 30,000 to 40,000 per $mm^3$, and the drug should be discontinued when counts fall to 20,000 per $mm^3$ or less. The leukocyte count continues to fall for another 2 to 3 weeks after the last dose. Failure to stop therapy before the count normalizes may result in severe (and possibly irreversible) marrow aplasia. The average patient requires 4 to 6 weeks of busulfan therapy to attain clinical remission; once the leukocyte count has leveled off, the patient may be given continuous maintenance therapy with low-dose busulfan or intermittent courses when the leukocyte count exceeds 30,000 per $mm^3$. In addition to its myelosuppressive effects, busulfan may produce pulmonary fibrosis or an addisonian-like syndrome characterized by hyperpigmentation, wasting, and hypotension.

#### Interferon-a

IFN-a appears to exert a direct antiproliferative effect against both normal and CML myeloid precursors, particularly those of the late progenitor compartment, which is preferentially expanded in CML. [118,119] Other effects include immunomodulation through increase in LFA-3 expression, [120] increase in ability of CML cells to attach to marrow stroma, [121] and modification of expression of HLA-DR antigens. [122] Because IFN-a does not appear to increase bone marrow fibrosis, the difficulties sometimes encountered in obtaining marrow aspirates from patients treated with this medication may reflect the increased cytoadhesion produced by IFN-a; “dry taps” may be avoided by discontinuing IFN-a for 3 to 7 days before the procedure. [121,123,124]

IFN-a is effective in reversing splenomegaly and normalizing the WBC and platelet counts. IFN-a–based protocols can achieve complete hematologic response (Table 21-7) in more than 80% of patients; approximately 5% to 30% of these patients will also achieve complete cytogenetic response (Ph $^1$-negative), while another 10% to 38% show a major cytogenetic response (less than 35% $Ph^1$-positive cells). Patients achieving a major cytogenetic response have projected 7 to 10-year survival rates of more than 80%. One-half of the patients who achieve long-term complete response may become pcr-negative for *bcr/abl*. [125]

| | White blood cell count | Splenomegaly | Percentage $Ph^1$+ cells (marrow)/(%) |
|---|---|---|---|
| Hematologic | | | |
| Complete | <9,000/mm³ (normal morphology) | None | |
| Partial | <20,000/mm³ (>50% decline) | Persistent | |
| Failure | >20,000/mm³ | Persistent | |
| Cytogenetic | | | |
| Complete | | | 0 |
| Partial | | | <35 |
| Minor | | | 35–95 |
| None | | | 100 |

**TABLE 21-7. RESPONSE CRITERIA FOR INTERFERON THERAPY OF CHRONIC MYELOCYTIC LEUKEMIA**

IFN-a appears to be most effective in the early phase of CML. The response rate declines dramatically in patients with long-standing disease (more than 1 year from diagnosis) or with accelerated or blast-phase progression. The addition of low-dose cytosine arabinoside improves the complete hematologic response rate (from 28% to 55%) for patients in the late chronic phase of CML, but not for those in accelerated phase. [126] When used as a single agent, IFN-a achieves a 12% greater 5-year survival than hydroxyurea (59% versus 47%).[127] For patients with good cytogenetic response (less than 35% $Ph^1$-positive metaphases), the actuarial survival at 5 years is in the range of 90%.[128]

In an effort to augment the therapeutic effects of IFN-a, various investigators have combined it with more conventional chemotherapeutic agents. The combination of busulfan and IFN-a produces severe myelosuppression and has not demonstrated significant benefit over IFN-a alone. [129] On the other hand, the combination of IFN-a and hydroxyurea has several clinical benefits, including higher hematologic response rate, rapid disease control with relatively high hematologic remission rate, low incidence of side effects, and longer duration of disease control; however, cytogenetic response rate is similar to that seen with IFN-a alone. [112] The combination of IFN-a with low-dose cytosine arabinoside appears to be superior to IFN-a alone with respect to complete cytogenetic response rate for patients treated in early chronic phase,[126] hematologic remission rate for patients in late chronic phase, [130] and overall survival rate.[131,132]

Because a small proportion of CML patients on IFN protocols are achieving long-term, event-free, pcr-negative survival, some authors are cautiously suggesting the possibility of cure with this regimen.[133] However, most patients with IFN-induced cytogenetic remissions still maintain demonstrable *bcr/abl* rearrangements.[134]

Enthusiasm for IFN-a as a first-line treatment for the chronic phase of CML must also be tempered by a recognition of its cost and potential toxicities. The most frequent side effect is a flulike syndrome (i.e., anorexia, fever, and liver dysfunction); neurologic, psychiatric, and dermatologic symptoms are also common. Occasionally, more severe toxicity, including thrombocytopenia, hypothyroidism with antithyroid antibodies, hemolytic anemia, and systemic lupus erythematosus, may develop.[135]

Tolerance to IFN-a may be improved by adherence to the following guidelines [124]:

1. Initiate therapy with hydroxyurea. Once the WBC count has been reduced below 10,000 to 20,000 per $mm^3$, IFN-a may be started and hydroxyurea gradually tapered.
2. IFN-a may be initiated at a lower dose for the first 3 to 7 days, then gradually escalated to full dose over the next 1 to 2 weeks. It should be administered at bedtime, and the patient may be premedicated with acetaminophen to minimize flulike symptoms.
3. Side effects, such as fatigue, depression, or insomnia, may be managed with a low dose of amitriptyline at bedtime.
4. Indications for dose reduction include: WBC count less than 2,000 per $mm^3$ or platelet count less than 50,000 per $mm^3$ and serious systemic toxicity.
5. Patients should continue IFN-a as long as a cytogenetic response persists or for at least 3 years beyond documentation of a complete cytogenetic response.
6. The serum half-life of IFN may be increased by attaching it covalently to polyethylene glycol (PEG). A phase I study showed PEG-IFN to be easier to administer (only once/week dosing), less toxic, and potentially more effective than standard IFN. [135a] Further studies are in progress to confirm these results and to determine the effectiveness of PEG-IFN in combination with cytarabine.

### Multiagent Chemotherapy

In rare cases, busulfan-induced bone marrow hypoplasia has produced reversion to $Ph^1$-negative status or to stable $Ph^1$-positive/$Ph^1$-negative mosaicism followed by prolonged remission.[136,137] Following up on this observation, several groups have used aggressive multiagent chemotherapy regimens (sometimes in conjunction with splenectomy) in an effort to ablate the $Ph^1$-positive clone.[138,139 and 140] In approximately one-half of the patients so treated, cytogenetic conversion of the marrow is achieved. The effect is usually of short duration, however, and has no significant impact on duration of chronic phase of survival.

### Splenic Irradiation

Irradiation of the spleen in patients with chronic-phase CML reduces splenomegaly, lowers the peripheral leukocyte count, and also reduces the number of immature cells and the mitotic index at sites distant from the spleen (e.g., bone marrow). This distant (abscopal) effect has been postulated to result from the release of an inhibitor into the plasma[141] or an interruption of the flow of CFU-GM from the splenic parenchyma into the circulation. [71] Before the introduction of busulfan, splenic irradiation was the main treatment for chronic-phase CML. Although symptomatic relief was achieved, disease control was generally short-lived (4 to 6 months), and the impact on survival was slight. Splenic irradiation was replaced by chemotherapy as the first-line treatment when a prospective, controlled clinical trial showed that intermittent splenic irradiation was inferior to busulfan in achieving consistent control of the leukocyte count and in prolonging survival. [142] Splenic irradiation may be considered for transient palliation of the symptoms produced by massive splenomegaly in patients refractory to systemic chemotherapy, but this treatment is generally ineffective and may result in profound myelosuppression.

### Splenectomy

The enlarged spleen of the patient with CML contains a substantial burden of leukemic cells, and, in some cases, blast transformation may originate in the spleen. [143] Investigators have proposed that removal of this pool of cells may delay metamorphosis. Several large controlled trials have failed to demonstrate any benefit from splenectomy in prolonging chronic phase or survival, however.[144,145] Splenectomy may be of benefit in selected patients with hypersplenism or painful splenomegaly; it may also be useful in reducing the leukemic burden in patients about to undergo ablative therapy before BMT. However, these potential benefits must be weighed against the risk of overwhelming postsplenectomy sepsis syndrome and extreme thrombocytosis.

### Bone Marrow Transplantation

The theoretical and technical aspects of BMT are discussed in Chapter 16. Three sources of hematopoietic stem cells have been used for transplantation in patients with CML patients: syngeneic (from an identical twin); allogeneic (from an HLA-identical sibling or matched unrelated donor); and autologous (from the patient).

***Syngeneic or Allogeneic Transplantation.*** Allogeneic BMT remains the only well-documented curative therapy for patients with CML. Disease status at the time of BMT has been considered to be the most powerful predictor of survival. In early studies, the best results had been obtained in patients who had undergone BMT in early first chronic phase (49% to 86% long-term survival); the chances for long-term survival became progressively worse when BMT is done in second chronic phase (30% to 58%), accelerated phase (15% to 35%), or blast phase (10% to 20%).[146,147 and 148] More recent studies have not confirmed that delaying transplant in chronic phase is necessarily an independent adverse prognostic factor. [149,150 and 151]

Some investigators have attempted to ameliorate the morbidity and mortality of graft-versus-host disease (GVHD) by depleting the donor marrow of T cells before engraftment. Various techniques have been used for this purpose, including purging with monoclonal antibodies, elutriation, and soybean lectin

agglutination/E-rosette depletion.[152,153,154,155,156,157,158,159] and [160] In general, T-cell depletion has reduced the incidence and severity of GVHD, but at the cost of an increase in the leukemia relapse rate, which negates any long-term survival advantage ( Table 21-8). Similarly, the use of autologous or syngeneic (identical twin) BMT has been accompanied by virtually no GVHD but a high leukemia recurrence rate. On the other hand, development of moderate-to-severe GVHD after administration of an allogeneic or unrelated donor graft appears to confer protection against relapse ( Table 21-8). These results indicate that the intense chemotherapy-radiotherapy conditioning regimens used in conjunction with BMT are often inadequate to eradicate the CML clone without augmentation by a graft-versus-leukemia (GVL) effect.

| Type of transplant | Relapse rate (%) |
|---|---|
| Autologous | 100 |
| T-cell depleted | 50–70 |
| Twin (syngeneic) | 40–70 |
| Allogeneic | 10–20 |
| Without GVHD | 11 |
| With GVHD | 5 |
| Unrelated | |
| Matched | 3 |
| Mismatched | 0 |

GVHD, graft-versus-host disease.
Data from Li JG. The leukocytopenic effect of focal splenic X-irradiation in leukaemic patients. Radiology 1963;80:871; Italian Cooperative Study Group on Chronic Myeloid Leukemia and Italian Group for Bone Marrow Transplantation. Monitoring treatment and survival in chronic myeloid leukemia. J Clin Oncol 1999;17:1838; Kantarjian HM, Giles FJ, O'Brien S, et al. Therapeutic choices in younger patients with chronic myelogenous leukemia. Cancer 2000;89:1647; Appelbaum FR, Jones L, Hale G, et al. Bone marrow transplantation for patients with chronic myeloid leukaemia: T-cell depletion with Campath-1 reduces the incidence of graft-versus-host disease but may increase the risk of leukaemic relapse. Bone Marrow Transplant 1996;17:53; and Arcese W, Sorrento M, Mauro FR, et al. Italian survey on allogeneic bone marrow transplantation for chronic myeloid leukemia. Bone Marrow Transplant 1989;4(Suppl 2):28.

**TABLE 21-8. RESULTS OF BONE MARROW TRANSPLANTATION IN CHRONIC MYELOCYTIC LEUKEMIA**

Fewer than 35% of patients with CML have an HLA-identical sibling, with perhaps another 5% having an acceptable, partially histocompatible related donor. For the remaining 60% to 70% of patients, HLA-matched unrelated donors present an alternative source of donor stem cells. Data from the National Marrow Donor Registry [161] indicate that a subset of patients with a combination of favorable prognostic features ( Table 21-9) can expect a 63% DFS at 3 years (DFS defined as lack of evidence of hematologic or bone marrow relapse). In a Seattle study, a similar favorable subset (<50 yr, HLA/DR matched, transplanted within 1 year of diagnosis) had a 74% probability of 5-year survival, an outcome comparable with recipients of fully matched sibling donor transplants. [161a]

| Variable | Relative risk of death |
|---|---|
| Acute GVHD <grade III | 0.36 |
| Chronic phase | 0.67 |
| Age (≤35 yr) | 0.69 |
| Early BMT (≤1 yr from diagnosis) | 0.72 |
| CMV seronegative recipient | 0.80 |

BMT, bone marrow transplant; CMV, cytomegalovirus; GVHD, graft-versus-host disease.
From McGlave PB, Bartsch G, Anasetti C, et al. Unrelated donor marrow transplantation for chronic myelogenous leukemia: initial experience of The National Marrow Donor Program. Blood 1993;81:543, with permission.

**TABLE 21-9. FAVORABLE RISK FACTORS FOR UNRELATED DONOR MARROW TRANSPLANTATION IN CHRONIC MYELOCYTIC LEUKEMIA**

Treatment options for patients who relapse after allogeneic BMT include use of IFN-a, [162] second transplants, or infusion of lymphocytes from the original donor, or both. Remissions may be induced by the use of IFN-a alone, but at least one-half of these patients ultimately have a relapse again. [163] The survival rate after a second BMT is poor and is complicated by increased procedure-related morbidity and mortality. [164,165] Patients with relapsed CML may also be restored to complete cytogenetic remission by administration of IFN-a together with leukocyte transfusions from the donor [166,167] or by the use of donor leukocyte infusions (DLIs) alone.[168] DLIs can induce second remission in up to 70% of patients; the probability of success is greater if the DLI is administered before hematologic relapse is evident. [169] The risk of GVHD is minimized if an escalating dose schedule of DLI is followed. [170,171] The durability of these remissions (which presumably result from a GVL effect) has not been established.

***Autologous Transplantation.*** Initial attempts at autologous BMT used marrow or peripheral blood cells collected from the patient during chronic phase and stored until there was evidence of metamorphosis. At that point, the patient received myeloablative chemotherapy or radiotherapy, followed by infusion of the chronic-phase cells, in an effort to restore a second chronic phase. This was, at best, a temporizing approach because the marrow was reconstituted with cells of the Ph $^{1}$-positive clone. Usually, the blast-phase cells proved resistant to even the most intensive regimens, and the second chronic phase was brief (median duration, 4 months); fewer than 30% of patients treated in this fashion survived for 1 year. [172,173]

Current approaches to autotransplantation center on the use of grafts that have been manipulated to purge Ph $^{1}$-positive stem cells and enrich for Ph$^{1}$-negative stem cells. Among the techniques currently used for purging are the following: incubation of the cells in long-term bone marrow culture to select for Ph $^{1}$-negative progenitors[174]; exploiting differential responses of normal versus Ph $^{1}$-positive clonogenic cells to various biologic agents (e.g., macrophage inflammatory protein-1a)[175]; separating cells based on differences in immunophenotype[176]; and harvesting peripheral blood stem cells during early hematopoietic recovery from intensive chemotherapy.[177] Carella and colleagues have developed a technique whereby Ph $^{1}$-negative cells are collected from the peripheral blood in the recovery phase after high-dose chemotherapy (idarubicin/cytarabine/etoposide) has been administered in conjunction with G-CSF. [178] The patient then is subjected to myeloablative therapy with busulfan and subsequently autografted with the previously harvested peripheral stem cells. A majority of such patients has achieved a Ph$^{1}$-negative state, which has been durable in some cases.

Preliminary results indicate that at least transient Ph $^{1}$ hematopoiesis can be achieved in 40% to 70% of patients treated with myeloablative therapy in chronic phase and reinfused with autologous marrow or peripheral blood preparations that have been treated to remove Ph $^{1}$-positive precursors.[174,176] Potential obstacles to maintaining permanent remission in these patients are as follows:

1. Contamination of the graft with residual Ph$^{1}$-positive stem cells: Studies using transduced gene markers indicate that Ph $^{1}$-positive cells persist in purged stem cell concentrates and are identifiable in clinical relapses. [179] This problem may be addressed by improved methods of selecting for Ph$^{1}$-negative stem cells.
2. Failure of the conditioning regimen to eradicate residual *in vivo* Ph$^{1}$-positive cells: This problem may be approached by performing BMT in early chronic phase, intensifying the preharvest chemotherapy regimen, and using a more rigorous myeloablative conditioning protocol.
3. Lack of GVL effect: This problem may be approached by the use of immunomodulatory agents such as IFN-a.[174]

***Detection of Residual Leukemia after Bone Marrow Transplant.*** Residual leukemic cells may be detected with increasing sensitivity at the morphologic (hematologic or bone marrow changes), cytogenetic (reappearance of the Ph $^{1}$ chromosome), or molecular level. Molecular relapses can be detected using RT-PCR analysis for *bcr/abl* mRNA transcripts or fluorescent *in situ* hybridization to assess interphase nuclei at the DNA level. More recently, molecular chimerism in myeloid (CD34$^{+}$) precursor cells and detection of p190 $^{bcr/abl}$ have emerged as novel markers of CML evolution that may identify impending cytogenetic relapses after BMT. [180]

Serial assessments of patients with CML have indicated that persistence or reappearance of Ph $^{1}$-positive cells does not necessarily indicate the inevitability of clinical relapse and an unfavorable outcome. Indeed, these patients may show various karyotypic responses after BMT, including (a) complete eradication of the Ph $^{1}$-positive clone, (b) cytogenetic relapse in the presence or absence of hematologic relapse, (c) transient reappearance of Ph $^{1}$-positive cells in the absence of hematologic

relapse, and (d) late (more than 1 year) disappearance of Ph[1]-positive cells in the absence of further therapy. [181,182]

Some investigators find PCR positivity to be limited primarily to patients studied in the first 12 months after BMT [183,184] or in patients given T-cell–depleted grafts, [185] whereas other investigators have found the majority of patients to be PCR positive regardless of the interval after BMT or the T-cell content of the graft. [186,187] and [188] Approximately 70% of patients are RT-PCR negative at 1 year posttransplant and remain so indefinitely. Ph [1] or PCR positivity up to 6 months after BMT may not have adverse prognostic significance, whereas persistence beyond this period identifies patients at a relatively high risk of relapse. [182,183] and [184,189] Predicting the prognosis for patients who vacillate between PCR-positive and PCR-negative status is more difficult. The transitory nature of Ph [1] or PCR positivity in some patients may reflect the persistence of a few long-living T or B lymphocytes or myeloid cells that lack proliferative potential; alternatively, these PCR-positive patients may have mechanisms (e.g., GVL effect) for continuous suppression of residual clonogenic leukemic cells *in vivo*.[190]

### Novel Strategies

The limited success, and excessive toxicity, of conventional chemotherapeutic, immunotherapeutic, and stem cell rescue techniques has stimulated the search for novel therapeutic approaches to the treatment of CML. Promising new approaches include the exploitation of the unique molecular biology of CML cells.

***Pharmacologic Inhibitors of Tyrosine Kinase Activity.*** A specific abl kinase inhibitor known as *STI 571* has demonstrated significant activity in chronic-phase CML patients, even in those who are refractory to interferon. [191] TKs function by transferring phosphate groups from adenosine triphosphate (ATP) to tyrosine residues on various substrates. STI 571 functions as a TK inhibitor by blocking the binding of ATP to *bcr/abl*, thereby inhibiting its TK activity. It is relatively selective in its activity, blocking all abl kinases (including p210 *bcr/abl*, p185 *bcr/abl*, v-*abl*, and the *c-abl*); however, it inhibits no other TKs (except for platelet-derived growth factor receptor and cKit kinase).[192,193] STI 571 also appears to be selectively toxic *in vitro* to cells expressing the *bcr/abl* TK. In a phase I trial, STI 571, as a single agent, induced cytogenetic responses in 45% of IFN-resistant chronic phase patients within 5 months of initiating therapy (10% of patients becoming 100% Ph-negative). [191] Responses were also achieved in 55% of patients with myeloid blast transformation (complete responses in 22%) and in 82% of patients with lymphoid blast crisis or Ph-positive ALL (55% complete responses).[194] All of the blast transformation patients subsequently relapsed, however, indicating the ability of CML cells to develop resistance to STI 571.

***Inhibition of Individual Signaling Pathways Downstream of* bcr/abl.** As the specific signal transduction events that contribute to leukemogenesis become elucidated, efforts may be made to develop rational therapeutic interventions designed to block these pathways. One example is the use of STI 571 to inhibit the TK activity of *bcr/abl* (discussed in the previous section). In addition, molecular strategies may be used to inhibit individual downstream mediators of the *bcr/abl* such as *ras*,[195] raf,[196] *jun* kinase,[197] and *myc*.[198]

***Immunotherapy.*** The junction site of *bcr* and abl generates a sequence of amino acids that is unique to the CML cell, thereby presenting a tumor-specific antigen that has the potential of activating an immune response. A *bcr/abl*-derived peptide vaccine that has demonstrated tumor-specific immunogenicity and a good safety profile is currently in clinical trials. [199]

### *Management of Blast Phase*

Blast phase is generally a treatment-resistant state that terminates fatally within weeks to months. Regimens developed for the treatment of acute myelogenous leukemia have proved disappointing in blast-phase CML (Table 21-10).[9,200,201,202,203,204,205,206,207,208,209] and [210] Patients with myeloblastic morphology have a median survival of 3 months after blast transformation. A regimen of alternate-day plicamycin (mithramycin) and daily hydroxyurea has been reported to convert myeloid blast phase back to chronic phase in occasional cases.[210] The subset of patients with lymphoblastic transformation is more sensitive to chemotherapy; approximately two-thirds of such patients revert to chronic phase after treatment with vincristine-prednisone regimens. [9] The response is of short duration (median less than 6 months), however, and fewer than 20% of patients survive for 1 year. The use of more intensive acute lymphoblastic leukemia regimens, such as the Memorial Hospital L10 or L10M protocols, may extend the median survival to 15 months.[197]

| Regimen | Number of patients | Complete response (%)[a] | Partial response (%)[b] | Reference |
|---|---|---|---|---|
| VCR/pred | 10 (myeloid) | 0 | NR | 95 |
| | 11 (lymphoid) | 67 | NR | |
| TRAMPCOL | 19 | 42 | 5 | 178 |
| HD ara-C | 21 | 24 | 13 | 179 |
| HU/6-MP/pred ±VCR/DNR | 202 | 12 | 22 | 180 |
| HU/6-MP/VP-16 | 5 | 20 | NR | 181 |
| L-10/L-10M | 15 (lymphoid) | 67 | NR | 182 |
| 5-aza/VP-16 | 27 | 4 | 56 | 183 |
| Mitoxantrone | 13 (myeloid) | 0 | 31 | 184-186 |
| DATA | 30 | 0 | 13 | 187 |
| Pica/HU | 6 (myeloid) | 100 | NR | 188 |
| | 3 (lymphoid) | 33 | NR | |

5-aza, 5-azacytidine; DATA, daunorubicin, 5-azacytidine, 6-thioguanine, cytosine arabinoside; HD ara-C, high-dose cytosine arabinoside; HU/6-MP/pred, hydroxyurea, 6-mercaptopurine, prednisone; HU/6-MP/VP-16, hydroxyurea, 6-mercaptopurine, VP-16; L-10/L-10M, vincristine, prednisone, doxorubicin, cyclophosphamide, methotrexate, cytosine arabinoside, L-asparaginase, thioguanine; plica, plicamycin; NR, not reported; TRAMPCOL, 6-thioguanine, daunorubicin, cytosine arabinoside, methotrexate, prednisone, cyclophosphamide, vincristine, L-asparaginase; VCR/DNR, vincristine, daunorubicin; VCR/pred, vincristine, prednisone.
[a]Reversion to chronic phase.
[b]Decline in blast count.

**TABLE 21-10. RESULTS OF CHEMOTHERAPY TREATMENT OF BLAST-PHASE CHRONIC MYELOCYTIC LEUKEMIA**

The results of BMT in blast phase are uniformly poor; fewer than 15% of patients achieve long-term survival with current regimens. In view of the dismal results achieved with other approaches, however, BMT is worth considering in selected cases.

## JUVENILE MYELOMONOCYTIC LEUKEMIA

As a result of review by the International Juvenile Myelomonocytic Leukemia (JMML) Working Group and the European Working Group of Myelodysplasia (EWOG-MDS) in Childhood, an international consensus has been reached whereby the condition known previously as JCML has been reclassified as JMML. [211,212] These groups have also suggested a group of minimal diagnostic criteria for the diagnosis of JMML (Table 21-11).

| Categories | Criteria |
|---|---|
| Suggestive clinical features | Hepatosplenomegaly |
| | Lymphadenopathy |
| | Pallor |
| | Fever |
| | Skin rash |
| Minimal laboratory criteria (must fulfill all three) | Peripheral monocytosis (>1 × $10^9$/L) |
| | Bone marrow blasts <20% |
| | Absence of Philadelphia chromosome and *bcr/abl* rearrangement |
| Further criteria (minimum of two required) | Elevated hemoglobin F (corrected for age) |
| | White blood cell count >10 × $10^9$/L |
| | Immature myeloid cells on peripheral smear |
| | Clonal cytogenetic abnormalities (including monosomy 7) |
| | Hypersensitivity of myeloid progenitors to granulocyte-monophage colony-stimulating factor (*in vitro*) |

**TABLE 21-11. DIAGNOSTIC CRITERIA FOR JUVENILE MYELOMONOCYTIC LEUKEMIA**

Like adult-type CML, JMML is a clonal panmyelopathy involving the pluripotent stem cell. [213] Individuals with neurofibromatosis type 1 (NF-1) are at particularly high risk for developing JMML (approximately 200 to 500 times the general population). [214] Monosomy 7, another myeloproliferative disorder of childhood, shares many of

the features of JMML, but it is usually associated with a lower level of hemoglobin F (Hgb F).[215,216] Although the exact relationship between these two conditions remains unresolved, both the International JMML Working Group and the EWOG-MDS have recommended that they be treated in a similar fashion.[217] It is more important to distinguish JMML from chronic viral infections (especially cytomegalovirus, Epstein-Barr virus, and human herpesvirus-6), which can also produce hepatosplenomegaly, lymphadenopathy, leukocytosis, thrombocytopenia, and elevated Hgb F levels. Serologic studies to detect an antibody response to one of these viruses are helpful. Bone marrow examination may be helpful in that viral infections may produce hemophagocytosis, which is not a feature of JMML. The distinctive granulocyte-macrophage colony-stimulating factor (GM-CSF) sensitivity manifested by JMML marrow cells in tissue culture (discussed in the following section) may also be useful in making the distinction.[218]

**Biology**

Hematologic and cytogenetic evidence confirms that JMML is a clonal disorder, with the leukemic progenitor cell capable of giving rise to erythroid, myeloid, monocytic, and megakaryocytic lineages[213,219,220 and 221] (and possibly lymphoid lineages as well). The predominant cell in the peripheral blood and the bone marrow appears to be a primitive monocytic precursor, however.[208,219,222,223] The high incidence of NF-1 and *ras* mutations in JMML (see Cytogenetic Features) suggest that deregulation of the *ras* signal transduction pathway may be a major mechanism in the pathogenesis of JMML. This may occur either via activating mutations of the *ras* genes or inactivating mutations in genes regulating *ras* (e.g., NF-1) whereby *ras* remains locked in an activated ATP-bound state, transmitting continuous signals for proliferation. A murine model using homozygous deletions of the NF-1 gene has confirmed the role of *ras* deregulation in promoting hypersensitivity to GM-CSF.[224]

A characteristic *in vitro* finding has been the excessive production of monocyte-macrophage colonies without a requirement for exogenous growth factors; this apparent spontaneous proliferation actually reflects an exquisite sensitivity of the JMML progenitor cells to low concentrations of various endogenous cytokines, including GM-CSF, tumor necrosis factor-a (TNF-a), and IL-1b.[225,226,227,228 and 229] Although it is not definite which of these molecules is the primary regulator of the JMML clone, some evidence suggests that autocrine secretion of TNF-a by the leukemic cells stimulates the production of GM-CSF by target cells, which, in turn, stimulates further proliferation and accumulation of leukemic monocytes and macrophages; IL-1 may represent an important accessory factor that augments the effects of TNF-a and GM-CSF.[227] TNF-a may augment the growth advantage of the malignant clone by inhibiting normal hematopoiesis.[228]

The exquisite sensitivity of the JMML clone to GM-CSF does not appear to result from abnormalities in the GM-CSF molecule or its receptor.[221] Instead, there appear to be aberrations in the *ras* pathway by which GM-CSF transmits growth signals to the nucleus.[229] This pathway may be inappropriately amplified either by mutations in *ras* genes themselves[230] or by defective regulation of *ras*. Neurofibromin, the protein that is altered in NF-1, is normally a GAP and thereby, a negative regulator of *ras*; loss of normal neurofibromin control of *ras* can result in unregulated myeloid proliferation.[231,232] This may account for the increased incidence of JCML in NF-1 patients.

In support of cytogenetic data, functional studies have also implicated the erythroid lineage within the JMML clone. Clonogenic cells of the erythroid series have been demonstrated in large numbers in both the peripheral blood and the bone marrow of patients with JMML.[233] As with myeloid progenitors, these burst-forming unit erythroid cells require minimal concentrations of their “poietin” (erythropoietin in this instance) for *in vitro* colony production.[216,233,234] Furthermore, constitutive expression of erythroid markers can be demonstrated in clonogenic cells of patients with JMML.[235]

**Clinical Features**

Most patients with JMML are diagnosed before the age of 2 years. Presenting physical findings include cutaneous lesions (e.g., eczema, xanthomata, and café-au-lait spots), lymphadenopathy, hepatosplenomegaly, and hemorrhagic manifestations. Respiratory symptoms (e.g., chronic tachypnea, cough, and expiratory wheezing) may be prominent.

**Laboratory Features**

The peripheral blood is characterized by anemia, leukocytosis, and thrombocytopenia. The leukocytosis is generally not as pronounced as in adult CML and is associated with a relatively high proportion of blasts (including normoblasts), lymphocytes, and monocytes. The bone marrow shows both erythroid and myeloid hyperplasia; immature cells of the monocytic series are prominent, and megakaryocytes are infrequent.

The erythrocytes show many features characteristic of fetal-type erythropoiesis, including high Hgb F level, fetal glycine-alanine ratio in the c chain of Hgb F, fetal-type glycolytic enzyme pattern, and low I antigen expression.[236,237] The LAP score is low in approximately 40% of patients.[11] Immunologic abnormalities include strikingly high immunoglobulin levels, high incidence of antibodies to nuclear antigen and immunoglobulin G,[238] and possible inability to control Epstein-Barr virus infection.[239]

**Cytogenetic Features**

The cytogenetic pattern of JMML is heterogeneous, the only consistent feature being absence of the Ph $^{1}$ chromosome. Abnormalities, when found, most commonly involve chromosomes 7 and 8.[213,239] Monosomy 7 is found in approximately 25% to 33% of JMML patients.

At the molecular level, gene changes are found quite frequently in JMML cells. Approximately 15% of JMML patients have clinical features of NF-1 with accompanying NF-1 mutations, whereas another 15% have NF-1 gene mutations in the absence of other manifestations of neurofibromatosis.[240] Approximately 30% of JMML patients have mutations in the *ras* genes, usually in codons 12 or 13 of N-*ras* or K-*ras*.[241] NF-1 and *ras* mutations do not occur concurrently in the same patient.[240]

**Natural History**

The course of JMML is quite variable and hard to predict. Some patients may experience relatively indolent disease with prolonged survival, whereas others progress to death from infection or other complications of bone marrow failure. Transformation to an acute leukemic blast crisis is an unusual event in JMML, occurring in approximately 15% of patients.[242] Overall median survival time for patients with JMML is less then 9 months. Prognosis varies with age at diagnosis, however; infants may survive for extended periods (mean 5-year survival is 67%), whereas children older than 1 year have virtually 0% long-term survival.[243] Most patients die of infection; fewer than 20% progress to a terminal blast phase. As in adult CML, the blast phase of JMML may be heterogeneous: cases of B-cell[244] and stem cell transformation have been reported. JMML may also terminate in an erythroleukemia-like phase, characterized by anemia, erythroblastosis, and megaloblastic erythroid hyperplasia of the bone marrow.[245]

**Therapy**

Generally, chemotherapy has been of limited value in JMML. Oral 6-mercaptopurine, either alone or in combination with subcutaneous cytarabine, has produced symptomatic relief in some patients,[246] but supportive care has been as effective as vigorous chemotherapy in most cases. In some cases, intensive multiagent chemotherapy (as used for treatment of acute nonlymphoid leukemias) has produced clinical remissions lasting as long as 27 months or longer.[247] However, intensive multiagent chemotherapy has generally been associated with high morbidity and generally short-lived remissions.[212,248] Thus, the First International Workshop on MDS in Childhood has recommended that such an approach should only be used in the setting of a pretransplant regimen.[217] The combination of hydroxyurea and IFN-a may also show activity in JMML.[249] Stem cell transplantation is currently the gold standard for treatment of JMML with overall 5-year survival rates in the range of 20% to 30%; the major barrier to cure with this modality has been the high relapse rate (greater than 50%).[217]

***Biologic Approaches to Juvenile Myelomonocytic Leukemia***

**Isotretinoin**

Isotretinoin (13-*cis*-retinoic acid) has been demonstrated to attenuate the *in vitro* “spontaneous” proliferation of monocyte precursors and their selective hypersensitivity to GM-CSF.[250] An overall response rate of 40 % to 50% has been reported in a phase II clinical trial using doses of *cis*-retinoic acid as high as 200 mg

per $m^2$.[251] However, sustained unmaintained responses are rare.

### Inhibitors of *ras* Farnesyl Protein Transferase

A critical prerequisite for *ras* function is localization to the inner leaflet of the plasma membrane; this can only occur if newly synthesized *ras* undergoes sequential posttranslational enzymatic processing, a process known as *prenylation*.[252] The initial and rate-limiting step in this sequence is catalyzed by the enzyme farnesyl protein transferase. Inhibitors of farnesyl protein transferase have shown the ability to down-regulate *ras* function by preventing proper localization of the ras protein; this results in an antiproliferative effect. [253] Preclinical studies have indicated that farnesyltransferase inhibitors are well tolerated in laboratory animals and cause regression in some tumors.[254]

### Other Approaches

Other novel biologic approaches that are specifically designed to target the molecular peculiarities of JMML cells include: analogues of GM-CSF that antagonize its effect on JMML cells[255] and GM-CSF/diphtheria toxin fusion molecules.[256]

## FAMILIAL CHRONIC MYELOCYTIC LEUKEMIA

A familial form of CML has been reported in at least three pairs of infant siblings. [257,258] This disorder is indistinguishable from JMML by standard clinical and laboratory criteria; however, its evolution is less predictable. In each of the families studied, one sibling died of progressive leukemia, and the other had long-term asymptomatic survival.

### Chronic Myelomonocytic Leukemia

Chronic myelomonocytic leukemia (CMML) is a rare disorder of childhood characterized by recurrent upper respiratory and pulmonary infections, anemia, unexplained monocytosis, neutropenia, thrombocytopenia, and progressive splenomegaly. [259,260 and 261] Atypical monocytoid cells with unipolar hairy projections are seen in the peripheral smear, and the bone marrow is hypercellular, with a high proportion of young myeloid and monocytoid forms. In some patients, the course of the disease may be relatively indolent, and aggressive chemotherapy may actually shorten survival by producing severe pancytopenia. Low-dose cytarabine has achieved complete remissions in some adults with CMML.[262]

## CHRONIC MONOCYTIC LEUKEMIA

Chronic monocytic leukemia is characterized by anemia, neutropenia, and thrombocytopenia in association with an increased number of mature monocytic elements in the blood and bone marrow. Extramedullary tissues, such as skin, gums, and viscera, may also be involved. Only a few cases of childhood chronic monocytic leukemia have been reported, and some of these may actually represent cases of acute monocytic leukemia, histiocytosis, or JMML. [263,264]

## CHRONIC LYMPHOCYTIC LEUKEMIA

CLL is a disease primarily of elderly adults; only rare cases have been reported in children. [265,266,267,268,269,270 and 271] The neoplastic cell appears to be a small B lymphocyte closely resembling the B cells residing in the mantle zone of secondary lymphoid follicles.

### Clinical and Laboratory Features

Presenting features include pallor, hepatosplenomegaly, and generalized lymphadenopathy. Hematologic findings include anemia, lymphocytosis, and infiltration of the bone marrow with small mature lymphoid cells. Lymph node architecture is obliterated by a diffuse population of small lymphocytes. Functional immunologic defects of both B-cell and T-cell populations are demonstrable. These include hypogammaglobulinemia, inadequate antibody response to antigenic stimuli, and decreased responsiveness to mitogens.

The characteristic phenotypic features of the CLL B lymphocyte is coexpression of CD5 and faint amounts of surface immunoglobulins (sIg). Monoclonality of the lymphoid population is demonstrable by analysis of membrane sIg and of immunoglobulin gene rearrangement. These techniques have been applied to only a handful of pediatric cases, however. [265,266 and 267]

### Cytogenetics

Common nonrandom cytogenetic abnormalities in adult CLL include trisomy 12, 14q+ translocations, trisomy 3, and abnormalities of chromosome 6. [272,273] In a pediatric case, t(2;14)(p13;q32) with breakpoints at or near the k light chain and heavy chain loci was reported.

### Therapy

Historically, CLL has been treated in a palliative fashion using alkylating agents (chlorambucil or cyclophosphamide), and sometimes steroids. Only two reported pediatric cases[258,261] have been treated in this fashion, and both patients responded well. At least two others [260,270] have had stable courses without any chemotherapy. New approaches have employed interferon-alpha, monoclonal antibodies (rituximab-anti CD20; Campath-1H–anti CD52). [274,275]

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22

# MYELOPROLIFERATIVE AND MYELODYSPLASTIC DISORDERS

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## INTRODUCTION

The vast majority of cases of myeloproliferative (MPS) and myelodysplastic (MDS) disorders occurs in the elderly, but can on occasion occur in childhood. These diseases represent a heterogeneous group of clonal stem cell disorders that are manifest as ineffective hematopoiesis and an increased risk of developing acute myeloid leukemia (AML).[1,2,3,4,5,6,7,8,9 and 10] In MDS, ineffective hematopoiesis results in progressive pancytopenias, whereas in the MPS disorders, at least initially, ineffective hematopoiesis is characterized by excessive peripheral blood counts. Another feature common to the MDS and MPS disorders has been a relative inability to cure patients with conventional agents. Although a number of nontransplant treatment strategies have been explored for these disorders, cure for these disorders has most often been achieved with myeloablative therapy followed by allogeneic stem cell transplantation.

As illustrated in Figure 22-1, it may be helpful to consider pediatric MPS and MDS syndromes in the following manner: The MPS disease seen most commonly in children is the adult form of chronic myelogenous leukemia (CML) characterized by the Philadelphia (Ph) chromosome ( *bcr/abl*-positive) (discussed in Chapter 21). Other forms of MPS that are seen with some frequency in adults are exceedingly rare in children. Therefore, polycythemia vera, essential thrombocythemia, and agnogenic myeloid metaplasia (AMM)/myelofibrosis are only briefly discussed in this chapter. Interested readers are referred to the cited references and adult textbooks for a more in-depth discussion of these disorders. The MDS syndromes of childhood can be considered in terms of de novo (primary) MDS or secondary MDS. The secondary MDS syndromes may be thought of in terms of MDS resulting from exposure to chemotherapy or radiation; secondary to congenital, constitutional disorders (e.g., Down syndrome); or the result of associated bone marrow failure syndromes (e.g., Fanconi anemia and congenital neutropenia). Finally, a group of disorders that “bridge” the MPS and MDS disorders is discussed. This group includes juvenile myelomonocytic leukemia (JMML), which, though rare, is among the most common of these disorders in children. Also included in this group is chronic myelomonocytic leukemia (CMML) in the proliferative phase, a disorder that is extremely rare in children.[11] Finally, although familial monosomy 7 may predispose patients to the development of myelodysplasia,[12] MDS and myeloproliferative disorders characterized by abnormalities in chromosome 7 (e.g., monosomy 7 syndrome) are no longer considered to be a distinct entity or represent a separate diagnostic category.

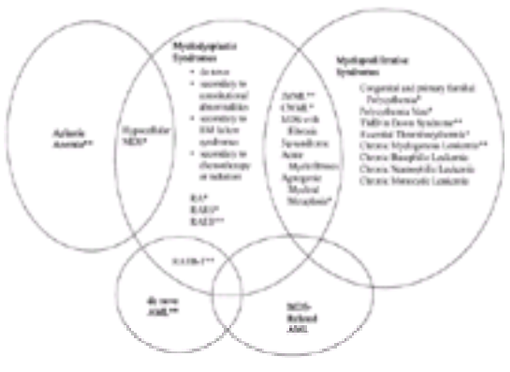

**FIGURE 22-1.** Conceptual model for myelodysplastic (MDS) and myeloproliferative (MPS) disorders. *Reported incidence. **Common form of MDS or MPS. AML, acute myeloid leukemia; BM, bone marrow; CMML, chronic myelomonocytic leukemia; JMML, juvenile myelomonocytic leukemia; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RAEB-T, refractory anemia with excess blasts in transformation; RARS, refractory anemia with ringed sideroblasts; TMD, transient myeloproliferative disorder.

## MYELODYSPLASTIC SYNDROMES

Until recently, the MDS syndromes of children, previously called *chronic monocytic leukemia*, *pre-leukemia*, and *hematopoietic dysplasia*, have been poorly defined, characterized, studied, and reported. In fact, one review of the classification of pediatric cancers failed to include MDS on its list of childhood malignancies.[13] This lack of understanding has been further complicated by the rarity of this disorder, lack of uniform diagnostic criteria, confusing nomenclature, and a poor understanding of the biologic mechanisms of disease. Further complicating a better understanding of these disorders in children has been the use of classification and prognostic systems designed for adult patients with MDS. These classification and prognostic systems, which have proven to be useful tools in the understanding and treatment of adult MDS, have proven to have little utility for children with MDS, in whom the pattern of disease, natural history of the disease, cytogenetic abnormalities, and associated anomalies are strikingly different from those of adults with MDS. As a result of these limitations, much of what has been described about childhood MDS has been generated from studies of adults with MDS, with the inclusion of small numbers of pediatric patients in these reports. Therefore, the accuracy and usefulness of much of the current information regarding children remains uncertain.

In recent years, however, many of these limitations have been addressed. International agreement has been reached with regard to uniform diagnostic criteria for many of these diseases, encompassing the use of morphology, immunohistochemistry, immunology, cytogenetics, and molecular biology. The number of scientists interested in childhood MDS has increased dramatically, and children with MDS are now being prospectively enrolled in large cooperative group clinical trials sponsored by the European Working Group on MDS of Childhood (EWOG-MDS), the Children's Oncology Group (COG), and others to help better define the true nature of these diseases and their response to uniformly delivered therapies.

## Definition

The MDS syndromes are a heterogeneous group of disorders of hematopoiesis that are characterized by ineffective hematopoiesis; impaired maturation of hematopoietic cells; progressive cytopenias associated with dysplastic changes in bone marrow cells; hypercellular, normocellular, or hypocellular bone marrow; and an increased risk of developing AML.

## Diagnosis

Morphologically, these disorders are characterized by dysplastic features of the peripheral blood and bone marrow in the granulocytic, megakaryocytic, monocytic, or erythrocytic lineages, occurring in a single lineage or in multiple lineages. However, precise definitions for the morphologic changes that constitute dysplastic changes have not been generally agreed on by hematopathologists. Although dysplastic features of marrow and peripheral blood cells are critically important in identifying MDS, dysplasia itself is not specific for MDS and can be the result of numerous other inciting events, unrelated to MDS. In fact, it has also been demonstrated that otherwise normal adults, with normal peripheral blood counts, who are donating bone marrow for allogeneic transplantation may have ringed sideroblasts and dysplastic features in granulocytes and erythroid cells. [14]

Dysplastic features in the granulocytic lineage of the bone marrow include dysgranulopoiesis with hypogranulation, nuclear hyposegmentation, megaloblastoid maturation, and a left shift with an increased number of myeloblasts. The peripheral blood may show dysgranulopoiesis with circulating myeloblasts, hypogranulation of neutrophils and eosinophils, and Pelger-Huet–type abnormalities. Dysplastic features of the bone marrow involving the erythroid lineage include megaloblastoid maturation, nuclear budding and multinucleated forms, and ringed sideroblasts. The peripheral blood may demonstrate dyserythropoiesis with polychromasia. Dysmegakaryocytopoiesis may be manifest in the bone marrow as micromegakaryocytes and abnormal megakaryocyte nuclei, with the peripheral blood demonstrating agranular and giant platelets. Dysplastic features of the monocyte lineage include an increase in bone marrow monocytes, abnormal granulation with persistence of azurophilic granules, hemophagocytosis, abnormal nuclei, and giant forms. The peripheral blood may demonstrate an increased number of monocytes and blasts forms.

Cytogenetic abnormalities may differ in patients with de novo MDS and those with therapy-related MDS. In children and adults with de novo MDS, cytogenetic analysis performed in a laboratory experienced in performing this test in malignant cells demonstrates clonal cytogenetic abnormalities in approximately 80% of patients[15,16] with an increasing percentage of patients with cytogenetic abnormalities as the disease progresses. [17] Most chromosomes have been reported to be involved in adults with MDS, although those most commonly involved are chromosomes 5, 7, 8, 9, 11, 12, 18, 19, 20, and 21. [18] Involvement of these chromosomes may involve complete or partial chromosome loss (e.g., monosomy 7 and 7q–, respectively), chromosome gain (e.g., trisomy 8), and various chromosome translocations. However, chromosome abnormalities, including t(8;21), t(15;17), t(9;11), and inv(16), commonly found in de novo AML, are not commonly seen in MDS. It is perhaps appropriate to consider a diagnosis of de novo AML with low blast count in children thought to have MDS characterized by chromosomal abnormalities commonly found in AML [e.g., t(8;21) and inv (16)].

Greater than 50% of children with de novo MDS (with a higher percentage in secondary MDS) have a detectable chromosome abnormality. The karyotypic abnormalities most commonly seen in de novo MDS in children include –7, 7q–, and +8. The addition of chromosomes 6, 9, and 11 as well as deletions of chromosomes 11, 12, and 13 are rarely present, but have been reported in pediatric MDS patients. Of importance is the finding that certain chromosomal abnormalities commonly present in de novo MDS in adults, including –5, 5q–, and –Y, are not present in pediatric MDS.

Karyotypic analysis is routinely performed using standard chromosome banding techniques. These techniques have significant limitations, including contracted metaphases, poor chromosome spreads, and limited ability to identify complex translocations and small insertions. Fluorescence *in situ* hybridization techniques have helped to overcome some of these technical limitations, [19] but are still limited to abnormalities that have been previously characterized. The recent development of multicolor spectral karyotyping may augment conventional banding techniques, as it increases the ability of cytogeneticists to identify unidentified chromosomal aberrations.[20,21] These technical advances may aid in the identification of significant, currently identified chromosomal abnormalities in children with MDS.

Flow cytometric analysis may also be used to gather additional evidence of dysplasia as determined by aberrant cell surface antigen expression. As an adjunct to morphology, flow cytometry can also be used to quantitate the percentage of myeloblasts and CD34 $^{+}$ cells in the marrow.[22] Additionally, in the evaluation of pediatric patients for the rare disease, paroxysmal nocturnal hemoglobinuria (PNH), flow cytometric analysis for the cell surface antigen CD91 (glycosylphosphatidylinositol-linked cell surface proteins), is a more sensitive and specific measure of PNH-like cells than the traditional Ham's test. [23] However, although many adult patients with MDS have been demonstrated to have PNH-like cells in all lineages, clinical evidence of hemolysis is not usually detectable. [23] Therefore, although flow cytometry may serve as a useful tool to aid in the diagnosis of MDS, current classification and prognostic criteria do not require flow cytometry results for classification or prognostication.

## Classification

A number of criteria for classifying MDS syndromes have been proposed. The most commonly used criteria for adults is that proposed by the French-American-British (FAB) Cooperative Group in 1982.[24,25] and [26] As is illustrated in Table 22-1, this classification recognizes five distinct forms of MDS: refractory anemia (RA), RA with ringed sideroblast (RARS), RA with excess blasts (RAEB), RA with excess blast in transformation (RAEB-T), and CMML. Some studies suggest additional subtypes of MDS in children and adults that do not easily fit into the current FAB classification. These include therapy-related MDS, hypoplastic MDS, refractory cytopenias with trilineage dysplasia, and MDS with associated myelofibrosis. [27,28,29,30,31] and [32] This classification system, though commonly used for children, has been of limited value in pediatric MDS patients. [33,34,35] and [36] The current FAB classification fails to account for MDS disorders associated with inherited conditions (e.g., certain congenital neutropenia, Shwachman syndrome, Down syndrome, type I neurofibromatosis, and Fanconi anemia), complex congenital abnormalities and the mitochondrial cytopathies.[37,38]

| Classification | Bone marrow blasts (%) | Peripheral blood blasts (%) | Sideroblasts (%) | Peripheral blood monocytes |
|---|---|---|---|---|
| Refractory anemia | <5 | ≤1 | ≤15 | ≤1 × 10⁹/L |
| Refractory anemia with ringed sideroblasts | <5 | ≤1 | >15 | ≤1 × 10⁹/L |
| Refractory anemia with excess blasts | 5–20 | ≤5 | Variable | ≤1 × 10⁹/L |
| Refractory anemia with excess blasts in transformation | 21–30 or Auer rods present | ≥5 or Auer rods present | Variable | Variable |
| Chronic myelomonocytic leukemia | ≤20 | <5 | Variable | ≥1 × 10⁹/L |

From Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 1982;51:189–199, with permission.

**TABLE 22-1. FRENCH-AMERICAN-BRITISH CLASSIFICATION OF MYELODYSPLASTIC SYNDROMES**

Using the FAB criteria, RA, RAEB, and RAEB-T have been reported in children, with RA being uncommon and RARS only rarely reported. [35,39,40,41,42,43] and [44] CMML is only rarely seen in children. CMML shares many characteristics with JMML. [11,40,45,46,47,48] and [49] However, the main distinguishing characteristic is the high percentage of fetal hemoglobin in JMML children. JMML is also most common in children younger than 5 years of age, whereas CMML is extremely rare in children younger than 5 years.

As a result of limitations in the FAB classification system, the World Health Organization (WHO) recently proposed changes to the FAB criteria. [50,51] Among the WHO's proposed changes are the elimination of the subtype RAEB-T, a reduction in the marrow blast count to 20% for a diagnosis of AML, the division of the RAEB subtype into RAEB-I (consisting of 5% to 10% marrow blasts) and RAEB-II (11% to 20% blasts), and removal of Auer rods as having any impact on the assignment of FAB

subtype. WHO also proposes the creation of two new subtypes for patients with refractory cytopenias with multilineage dysplasia (excluding erythroid dysplasia and less than 5% blasts) and a new category for unclassified MDS, including severe myelofibrosis, 5q–, 17p1, etc. ( Table 22-2). Finally, CMML is removed from the FAB schema and reclassified as a MPS. Although this new classification of CMML accounts for the proliferative phase of CMML, it does not allow for classification of the nonproliferative phase of the disease. These proposed changes remain untested and are considered to be controversial. Their utility in pediatric MDS remains unclear.

| |
|---|
| Refractory anemia |
| With ringed sideroblasts |
| Without ringed sideroblasts |
| Refractory cytopenia with multilineage dysplasia |
| Refractory anemia with excess blasts |
| 5q– syndrome |
| Myelodysplastic syndrome, unclassifiable |

[a]Note: Refractory anemia with excess blasts in transformation is now classified as acute myeloid leukemia.

**TABLE 22-2. PROPOSED WORLD HEALTH ORGANIZATION CLASSIFICATION OF MYELODYSPLASTIC SYNDROMES[a]**

## Incidence and Epidemiology

Despite an increasing number of case reports, the true incidence of pediatric MDS is unknown, but generally is estimated to account for approximately 3% to 7% of childhood hematologic malignancies. Only three actual population-based studies have been reported. [52,53 and 54] In the study based in Denmark, an annual incidence of 4.0 cases per million was determined, representing 9% of all pediatric hematologic malignancies in that country. More recently, the annual incidence of pediatric MDS in British Columbia, Canada, was determined to be 3.1 cases per million, representing 6% of hematologic malignancies in British Columbia. [54] In contrast, data from the Surveillance, Epidemiology, and End Results survey for the years 1990 to 1995 suggest an annual incidence for de novo AML in children age 15 years or younger is 7.0 cases per million.[55] Most observers think that pediatric MDS has an incidence of approximately 10% of that of AML. Hence, if this estimate is accurate, the annual incidence of MDS in the United States may be in the range of 0.8 cases per million.

A number of environmental exposures and genetic disorders may predispose patients to the development of MDS. Exposure to alkylating agents (e.g., cyclophosphamide and nitrogen mustard),[56,57] topoisomerase II agonist and antagonist (e.g., etoposide), and ionizing radiation may increase the probability of MDS and AML. Many children with MDS have other associated anomalies, with Down syndrome being seen most frequently. Other congenital syndromes, such as neurofibromatosis and bone marrow failure syndromes, including Kostmann's congenital neutropenia, Schwachman syndrome, Blackfan-Diamond syndrome, thrombocytopenia with absent radii, trisomy D, Klinefelter's syndrome, familial thrombocytopenia, and Fanconi anemia, have been demonstrated to predispose these children to an increased probability of MDS and subsequent AML. [55,58]

## Etiology and Pathophysiology

The etiology of MDS is not well understood. The inciting events in MDS have been difficult to discern. This is in part due to significant heterogeneity of these diseases as well as a poor understanding of the numerous molecular and cellular abnormalities likely responsible for initiation of the disease versus those abnormalities that are secondary to genetic instability in MDS clones. Like Ph chromosome–positive ($Ph^+$) CML, MDS is thought to originate in a single immature hematopoietic stem cell. However, unlike CML, in which the basic molecular defect (*bcr/abl* translocation) has been identified, a single molecular abnormality has not been identified in MDS. Evidence for involvement of an immature hematopoietic stem cell with subsequent clonal involvement of multiple lineages was first suggested in studies of adult females with MDS using a marker of X-chromosome inactivation, glucose-6-phosphate dehydrogenase isoenzymes,[59,60] and cytogenetic marker studies.[2,61,62,63,64,65 and 66] Newer assays to determine clonality, including X-linked restriction fragment polymorphisms, polymerase chain reaction of the phosphoglycerate kinase gene, and X-chromosome human androgen receptor locus assays, demonstrate the clonal involvement of granulocytes and erythrocytes, [4,67] with possible, but still controversial involvement of the B- and T-lymphocyte lineages.[5,68,69,70,71,72 and 73] It should be noted, however, that these studies have not been widely performed in pediatric MDS patients. Most studies of clonality in MDS have been performed in older adults in whom clonal and oligoclonal hematopoiesis may occur, even in apparently normal individuals.[74,75] Additionally, to account for heavy Lyonization with skewing of patterns of clonality, normal, uninvolved somatic tissues (e.g., skin fibroblasts) have not always been used as appropriate negative controls in these studies, [76] making interpretation of the results difficult. Despite these limitations, however, clonality appears to be an early event in the origin of MDS.

It is generally thought that MDS involves an abnormality in an immature stem cell that leads to the proliferation of a MDS clone of cells along with normal hematopoietic elements. This mosaicism of normal and abnormal hematopoiesis may coexist for prolonged periods. [77] However, as additional injuries and molecular defects occur in this abnormal, MDS clone, the abnormal clone appears to develop a competitive advantage over normal hematopoietic elements, leading to suppression of normal hematopoiesis[70,73,78] and eventually to only ineffective, clonal hematopoiesis. [2,79,80] As these MDS cells may have maturation defects and normal hematopoiesis is impaired, peripheral cytopenias eventually occur. As additional defects are acquired (e.g., karyotypic abnormalities, *ras* gene mutations, *FHIT* gene alterations, mutations in *WT1*, and *p53* gene abnormalities) as the result of additional toxic exposures or as a result of a generalized genetic instability in these abnormal clones, transformation to AML may occur.[2,79,80,81,82,83,84,85,86,87 and 88]

A number of distinct cytogenetic abnormalities may occur as these abnormal clones evolve. The most common abnormalities include the complete loss of specific chromosomes (e.g., chromosome 7), partial chromosome losses [e.g., the long arm of chromosomes 7 (7q–)] or the addition of extra chromosomes (e.g., trisomy 8).[62,64,89,90,91,92,93,94,95,96,97,98,99,100,101,102,103,104,105 and 106] It is not known if abnormalities in these chromosomes are inciting events leading to the development of MDS or secondary events. The biologic implications of these chromosomal abnormalities are also unclear. It is known, however, that a number of genes presumed to be important in the control of hematopoiesis are encoded on these large areas of DNA that are gained or lost during the evolution of MDS clones. For example, chromosome 5q, commonly seen in older adult females with 5q– syndrome but rarely seen in pediatric MDS, contains genes encoding for granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage CSF, interleukin-3 (IL-3), IL-4, and IL-5, erythrocyte glutathione reductase-1, and interferon regulatory factor 1. [107,108 and 109] Many of these genes have significant roles in the regulation of hematopoiesis. The clinical impact of abnormalities to these genes is not well described. For childhood MDS, little is known of the genes involved in abnormalities of chromosome 7, the most commonly described karyotypic abnormality in children. However, it has been suggested that MDS characterized by familial monosomy 7 or 7q– may be the result of multiple genetic events because the predisposition to MDS is not located within a consistently deleted segment on the long arm of chromosome 7.[12]

Additionally, although the genes responsible for MDS have not been clearly identified, it has been postulated that molecular abnormalities in certain tumor suppressor genes, also located on these involved chromosomes, may be responsible for the evolution of the MDS clones to overt AML. [110] For example, children with inherited abnormalities of the neurofibromatosis type 1 (*NF1)* gene are at increased risk of MDS, MPS, and AML after loss of the normal allele. However, loss of heterozygosity has not been found in adults with MDS.[111] Loss of heterozygosity of *NF1* or in other growth regulatory and tumor suppressor genes has not yet been demonstrated in children or adults.[112,113 and 114]

Finally, whereas mutations in the proto-oncogene *ras* have been reported in many human malignancies, including 30% to 40% of children with MDS, the true frequency of point mutations in *ras* is uncertain.[115,116,117 and 118] It has been demonstrated that an increasing frequency of *ras* mutations occurs with progression of MDS,[119,120] but it is not currently thought that activating point mutations in *ras* alone is sufficient for the initiation of MDS. Rather, it is currently believed that *ras* mutations represent a later, secondary event in the progression of MDS and are the result of an overall genetic instability inherent to MDS clones.

Based on these data, it has been suggested that MDS represents a hyperproliferative disorder that is initially held in check by apoptosis mechanisms, perhaps induced by events that cause DNA damage that results in cell cycle arrest. [121] Progression of MDS and transformation to AML occur as the genetically unstable clones

develop additional molecular abnormalities. This same multistep pathogenesis has been implicated in the tumorigenesis of adult carcinomas.

Thus, it may be very important to differentiate truly de novo AML, in which the blast count is irrelevant, from that of MDS-related disease. [121] In this model, RA and RAEB may be indicative of MDS, whereas RAEB-T may be truly de novo AML with a lower blast count. Differentiation between diseases in these two categories is very important as these diseases may respond to different therapies. Although this model may accurately reflect the mechanism of disease for truly de novo AML, MDS, and MDS-related AML in the elderly, it remains unclear if this mechanism represents operant mechanisms for de novo MDS in children. [121,122]

**Clinical Presentation**

The presenting signs and symptoms of MDS in children are nonspecific and are usually the result of pancytopenia. However, it is not unusual for abnormalities in the peripheral blood to be determined on routine health screens in asymptomatic children. Symptomatic children will frequently present with signs of marrow failure, including pallor, fatigue, bruising, petechiae, and infections. In addition, infectious complications in patients whose MDS is characterized by monosomy 7 may be due, in part, to defects in neutrophil chemokinesis and chemotaxis. [123,124] Although common in the MPS syndromes, lymphadenopathy, hepatomegaly, and splenomegaly are unusual presenting signs for children with MDS.

**Differential Diagnosis**

A careful history, physical examination, and evaluation of the peripheral smear and bone marrow for dysplastic features and clonal cytogenetic abnormalities easily identify most cases of MDS in children. However, other diseases must be considered in the differential diagnosis, particularly when the diagnosis is complicated by an absence of clonal cytogenetic abnormalities and an aplastic marrow. The possibility of various associated anomalies, such as Down syndrome, and congenital marrow failure syndromes, such as congenital neutropenia, Fanconi anemia, Shwachman syndrome, Blackfan-Diamond syndrome, the congenital dyserythropoietic anemias, and hereditary sideroblastic anemia, should be considered. Severe aplastic anemia (SAA) can be difficult to distinguish from hypoplastic MDS. While rare, PNH can occur in childhood and should be considered. However, as PNH-like cells have been reported in MDS, care should be taken in the evaluation of patients for PNH. [23] Certain nutritional deficiencies, including vitamin $B_{12}$ and folate deficiency can result in megaloblastoid changes that appear similar to dysplastic changes present in MDS. Other nutritional deficiencies of iron, riboflavin, pyridoxine, and thiamine [125] should also be considered. The differential diagnosis should also include AML with low blasts counts,[126] MPS disorders, mitochondrial cytopathies, viral infections (e.g., human immunodeficiency virus, [127,128] parvovirus, Epstein-Barr virus, human herpes virus 6, and cytomegalovirus), toxins (e.g., insecticides and chemotherapy), and changes secondary to cytokine and radiation exposures.

**Treatment and Outcome**

Given the clonal origin of MDS in an immature hematopoietic stem cell, these diseases are generally thought to be incurable with conventional therapies. Although a number of supportive care measures, chemotherapy combinations, hematopoietic growth factors, hormones, immune modulators, and differentiating agents have been studied, cures are generally achieved only after myeloablative chemotherapy and radiation with allogeneic transplantation.

Supportive care approaches have been attempted in some children with RA. Although the natural history of RA has not been well described in children, long-term survival has been seen in some children with RA who receive only supportive care. In addition, rare MDS patients whose disease is characterized by the presence of monosomy 7 have achieved spontaneous hematologic and cytogenetic remissions.[129]

Hormones (e.g., glucocorticoids, androgens, and danazol), [130,131,132,133 and 134] differentiating agents (e.g., 13-*cis*-retinoic acid, all-*trans* retinoic acid), [135,136,137,138 and 139] and hematopoietic growth factors (e.g., GM-CSF, granulocyte colony stimulating factor, and erythropoietin) [140,141,142,143 and 144] have been studied extensively in adults with MDS, generally with results that do not support their role in the routine management of MDS patients. The role of these agents in MDS of childhood has been largely untested.

Amifostine, a phosphorylated aminothiol, has been shown to protect hematopoietic progenitor cells and other normal tissues from the toxic effects of alkylator and organoplatinums while maintaining the antitumor effects of these agents. [145,146 and 147] Although the mechanism of chemoprotection in normal cells is not clearly understood, amifostine is thought to be mediated through oxygen-free radical scavenging pathways. [148] *In vitro* studies of amifostine and MDS cells have demonstrated that exposure to amifostine enhances the survival of primitive hematopoietic progenitors, [149] increases the numbers of normal colonies with decreasing colonies derived from abnormal progenitors,[150] and reduces the proportion of apoptotic early progenitor cells. [151] Amifostine has been studied in phase I and phase II studies in adults with MDS.[152,153,154 and 155] These studies have suggested that amifostine may be effective in ameliorating MDS-induced cytopenias in a portion of patients. In the largest of these adult studies, single or multilineage cytopenias were improved in 41% of patients, with a decrease in bone marrow blasts in 35% of patients. [155] Amifostine has been used in conjunction with melphalan for children with refractory malignancies as part of a phase I, dose escalation trial. [156] Dose-limiting toxicities were not encountered, with transient hypotension, flushing, nausea, and vomiting seen as non–dose-limiting toxicities. These results in adults with MDS and children with other malignancies are encouraging. However, there are currently no data on the use of amifostine for the treatment in children with MDS.

Low-dose chemotherapy approaches have been variably explored in both children and adults with MDS based on the theory that they may play some role in the differentiation of the malignant clone. Agents tested include cytarabine, melphalan, hydroxyurea, etoposide, topotecan, 5'-azacytidine, 6-mercaptopurine, and busulfan.[11,157,158,159 and 160] Most of these low-dose approaches to therapy have resulted in only partial and temporary responses in a small portion of patients. Most observers believe that the role of low-dose chemotherapy is only in reducing toxicity, with a concomitant lowering of efficacy. Therefore, the use of low-dose chemotherapy is generally not thought to be of particular benefit in the treatment of children with MDS.

Intensive, AML-like chemotherapy has been investigated in the setting of adult MDS [161,162,163,164,165 and 166] and pediatric MDS with and without subsequent hematopoietic stem cell transplantation.[42,43,165,167,168] Results from these studies demonstrate conflicting results with regard to the efficacy of high-dose, intensive therapy. There are, however, accumulating data that some forms of MDS (i.e., RAEB-T) do respond to chemotherapy, both in adults and children, like patients with AML. Overall, however, the role of AML-like intensive chemotherapy with or without subsequent stem cell transplantation and its applicability to subgroups of MDS patients are unclear, are considered to be controversial, and are being prospectively studied. [168]

Allogeneic hematopoietic stem cell transplantation was initially attempted in children and adults with MDS as a result of the inability of conventional forms of therapy to cure these diseases.[170,171,172,173,174,175 and 176] Before 1990, there were few reports of the efficacy of this approach. Since 1990, there have been a number of reports demonstrating the success of allogeneic transplantation for the treatment of children and adults with MDS. [177,178,179,180,181,182,183,184,185,186,187,188,189,190,191,192,193 and 194] Despite this proliferation of published transplant results, most of the studies include pediatric patients only as a part of larger adults series, thereby making it difficult to determine overall results for children. Several reports specific for pediatric patients have recently been published. [180,193,195,196,197 and 198] Although these reports are helpful in that they exclude adults, these studies are complicated by very small numbers of patients and the inclusion of children with other diseases, including SAA or AML. Therefore, information is generally lacking for allogeneic transplantation in children, aside from that available in larger series of patients that include both children and adults. Despite this, stem cell transplantation is generally considered the treatment of choice for children with the proliferative forms of MDS, particularly for children with HLA-matched related donors.

The largest reported series of children and adults with MDS was reported by investigators at the Fred Hutchinson Cancer Research Center in Seattle. [189] Between 1981 and 1996, 251 children and adults were transplanted from an HLA matched related donor (n = 147), unrelated donor (n = 70), or HLA-mismatched related donor (n = 34). Patients had a median age of 38 years (range, 1 to 66 years) with a median time from diagnosis to transplant of 8 months (range, 1 to 192 months). One hundred and forty-four patients had advanced disease (i.e., RAEB, RAEB-T, or CMML), 107 had less advanced disease (i.e., RA or RARS), and 36 had therapy-related MDS. Using the criteria of the International Prognostic Scoring System (IPSS) for MDS ( Table 22-3, Table 22-4 and Table 22-5, Fig. 22-2),[199] cytogenetic results were available to classify 241 of these patients into risk-based categories. One hundred and ten patients were classified as good-risk, 53 intermediate-risk, and 78 were poor-risk. Various preparative regimens were utilized, with 172 patients receiving a total body irradiation (TBI)–based regimen and 79 receiving a busulfan-based preparative regimen. At a median follow-up of 3.7 years, the 3-year cumulative incidence of non-relapse mortality (NRM) was 42%, with longer disease duration and older age being most predictive of NRM. The 3-year cumulative incidence of relapse was 17%, with advanced disease, poor-risk cytogenetics, and transplantation among the earlier cohorts of patients being most predictive of relapse. Three-year actuarial disease-free survival (DFS) was 41%, with younger age, less advanced morphology, and good-risk cytogenetics predictive of better survival. Similar results have been reported by other groups. [179,200,201]

| Variable | Score | | | | |
|---|---|---|---|---|---|
| | 0 | 0.5 | 1.0 | 1.5 | 2.0 |
| Bone marrow blasts | <5% | 5–10% | — | 11–20% | 21–30% |
| Karyotype | Good | Intermediate | Poor | — | — |
| Cytopenia[a] | 0 or 1 lineage | 2 or 3 lineages | — | — | — |

[a]Hemoglobin <10 g per dL, platelet count <100,000 per μL, absolute matrophil count <1,500 per μL.
From Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes [Published erratum appears in Blood 1998;91:1100]. Blood 1997;89:2079–2088, with permission.

**TABLE 22-3. INTERNATIONAL PROGNOSTIC SCORING SYSTEM FOR MYELODYSPLASTIC SYNDROMES**

| Classification | Karyotype |
|---|---|
| Good | Del (5q) |
| | Del (20q) |
| | -Y |
| Poor | Complex (≥3 abnormalities) |
| | Chromosome 7 abnormalities |
| Intermediate | All other abnormalities |

**TABLE 22-4. CLASSIFICATION OF KARYOTYPES**

| Risk group | Score |
|---|---|
| Low | 0 |
| Intermediate 1 | 0.5–1.0 |
| Intermediate 2 | 1.5–2.0 |
| High | ≥2.5 |

**TABLE 22-5. INTERNATIONAL PROGNOSTIC SCORING SYSTEM RISK GROUPS**

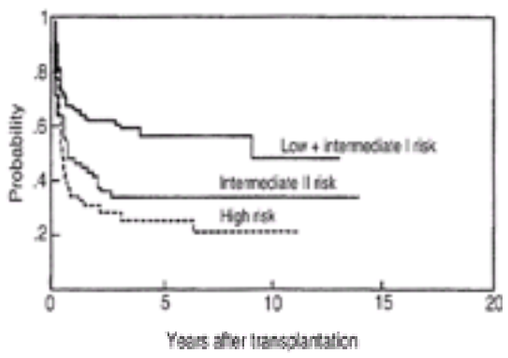


**FIGURE 22-2.** Impact of International Prognostic Scoring System on disease-free survival for transplanted myelodysplastic patients. [From Appelbaum FR, Anderson J. Allogeneic bone marrow transplantation for myelodysplastic syndrome: outcomes analysis according to IPSS score. Leukemia 1998;12(Suppl 1):S25–S29, with permission.]

Most of the patients transplanted for MDS have received bone marrow grafts from HLA-matched related donors. However, given the fact that HLA-matched related donors are available for only a minority of patients in need of allogeneic transplantation, the use of alternative donors has been increasingly utilized. [193,196,200,202,203,204 and 205] The largest series of unrelated bone marrow donors for the transplantation of children and adults with MDS and MDS-related AML was reported by the Seattle group.[184] These results were strikingly similar to those previously reported for the larger series of patients transplanted from unrelated donors, HLA-matched related donors, and HLA-mismatched related donors.[189] A 2-year actuarial probability of NRM was 48%, with older age and longer duration of disease most predictive of transplanted-related death. The 2-year actuarial probability of relapse was 28%, with higher relapse rates in patients with MDS-related AML and RAEB-T. The 2-year actuarial probability of DFS for this series of patients was 38%. Other reports of unrelated donor transplants in similar patient populations [200,203,204] have also shown high probabilities of NRM and relatively low DFS.

A variety of preparative regimens have been used in the transplantation of children and adults with MDS. Most of these regimens are based on the use of TBI with cyclophosphamide or busulfan and cyclophosphamide. Although comparative studies of these regimens for children have not been performed, larger studies inclusive of children and adults suggest similar results for these regimens, particularly for less advanced diseases. [180,183]

The timing of allogeneic transplantation for the treatment of children with MDS has not been well defined. The difficulty in ascertaining the most appropriate timing of transplantation is related to uncertainty about the natural history of childhood MDS, particularly in lower risk groups (e.g., RA), as well as significant toxicity associated with the transplant procedure. The decision to proceed with allogeneic transplant must take into account the disease morphology, cytogenetic abnormalities, and potential stem cell sources.

As there are little published data in children, it is considered reasonable to observe children with RA who are not having infectious complications or are transfusion independent until progression of their disease, until they are at risk of infectious complications as a result of neutropenia, or until they become transfusion dependent. It is also generally recommended that children with “symptomatic” RA and RAEB with a matched-related stem cell donor proceed to transplantation as soon as possible with consideration for an alternate donor transplant if matched-related donor is not available. Because RAEB-T may represent AML with low blast count, it may respond to AML-like chemotherapy. It is recommended that children with RAEB-T receive AML chemotherapy as initial therapy with transplantation if a matched-related donor is available. Consideration for alternate donor transplantation occurs when chemotherapy fails. Although the IPSS may be useful in helping to

determine the rapidity with which adult patients with MDS should proceed to allogeneic transplantation, [189] the role of the IPSS in determining which children should be observed and which should proceed more rapidly to transplantation has not yet been determined.

### Prognosis

The FAB classification system for MDS alone has not been shown to be a reliable method of predicting the outcome of patients with MDS, even in adults. Therefore, a large number of widely disparate methods have been proposed for evaluating the outcome of patients with MDS. [16,25,42,199,206,207,208,209,210,211,212,213 and 214] These methods have variably included features such as age, morphology according to FAB classification, the percentage of bone marrow blasts, cytopenias, karyotype, lactate dehydrogenase levels, and numerous other features.[16,25,42,199,206,207,208,209,210,211,212,213 and 214] However, many of these methods for predicting clinical outcome have suffered from a lack of consensus on the nomenclature and definitions of MDS and insufficient sensitivity or specificity, thereby severely limiting their effectiveness. Many of these problems have been addressed in the proposed IPSS.[199] As illustrated in Table 22-3, Table 22-4 and Table 22-5, this method uses the percentage bone marrow blasts, karyotype, and the number of cytopenias to assign a score value. Based on the score value, patients are placed into one of four risk subgroups (i.e., low, intermediate-1, intermediate-2, and high) with further stratification based on age (e.g., 60 years old or younger and older than 60 years) ( Fig. 22-3). Proportional hazards analysis using the IPSS has suggested that blast percentage is the best predictor of outcome, followed by cytogenetic abnormalities, age, and gender. Compared to prior methods, the IPSS is increasingly used for adult MDS patients, as it appears to more accurately predict outcome for adult MDS patients. [199] However, this study was comprised primarily of elderly adult patients. Its use for pediatric patients is limited, as it fails to account for cytogenetic abnormalities common in the pediatric forms of MDS and associated congenital anomalies. Therefore, the relevance of this risk-based method of prognosis for children with MDS is unknown, but unlikely to be beneficial.

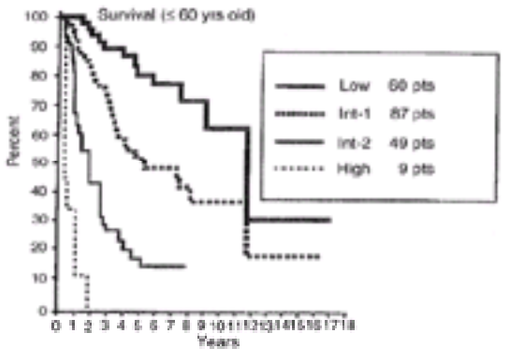


**FIGURE 22-3.** Survival according to age and International Prognostic Scoring System risk group for 816 patients with primary myelodysplastic syndrome. [From Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes (Published erratum appears in Blood 1998;91:1100). Blood 1997;89:2079–2088, with permission.]

Few risk-based classification schemas specific for pediatric MDS have been developed and prospectively validated. The lack of consensus on nomenclature and the rarity of MDS in pediatric MDS patients has greatly inhibited the development of an accurate method with which to determine clinical outcome. One attempt to create a pediatric-specific risk-based classification was recently proposed. [42] This method uses a modified FAB classification, platelet count, fetal hemoglobin level [hemoglobin F (HbF)], and cytogenetic complexity score to generate a pediatric scoring system [pediatric FPC (Hb *F-p*latelets-*c*ytogenetics)] to assign a score value. Validation of this method in a single institution suggested that this method of risk-based classification was highly predictive of survival for pediatric MDS patients. [42] The accuracy and usefulness of the pediatric FPC scoring system can only be determined in larger, prospective studies.

### Hypoplastic Myelodysplastic Syndrome

Despite significant progress in understanding the cellular and molecular properties of MDS, it continues to be very difficult to distinguish hypoplastic MDS from SAA. The disorder called *hypoplastic MDS* is not included in the FAB classification of MDS. In fact, the original classification schema required at least a normocellular or hypercellular marrow for a diagnosis of MDS.[25] It has been observed that some patients with SAA may develop dysplastic peripheral blood and marrow features, including dyserythropoiesis, megaloblastic changes, dysgranulopoiesis, ringed sideroblasts, atypical monocytes, PNH-like cells, and elevated fetal hemoglobin levels, particularly in the regenerative phase of the disease. [215] Various groups have also shown that some patients with SAA may have clonal hematopoiesis. [216,217] In addition, a significant portion (e.g., 10% to 30%) of patients with SAA eventually develop clonal hematopoietic abnormalities, including MDS, AML, and PNH. [218,219,220 and 221] Furthermore, it has also been observed that some patients with MDS respond to immunosuppressive therapy analogous to that used to treat SAA. [222,223 and 224] It therefore, remains very difficult for hematopathologists and clinicians to distinguish MDS with a hypocellular marrow from SAA. Many clinical and laboratory features must be taken into account in the diagnostic process, including the presence of conditions that have a predisposition to develop into MDS (e.g., congenital neutropenia, Fanconi anemia, PNH, and Shwachman-Diamond syndrome), morphology, single or multilineage dysplasia, reticulocyte counts, immunology, and cytogenetic abnormalities.

Some investigators have sought to use magnetic resonance imaging (MRI) as a tool to discriminate these disorders. MRI has a characteristic appearance in aplastic anemia by virtue of the absence of cellular elements and the presence of fatty marrow. [225,226,227,228,229 and 230] MRI is also able to identify areas of focal or diffuse areas of increased cellularity in adult patients with a diagnosis of MDS. [230,231] However, although MRI may be an aid in the identification of area of cellularity in an otherwise aplastic marrow, MRI alone is not sufficient to distinguish between SAA and MDS.

Accurate discrimination between SAA and MDS has tremendous prognostic and therapeutic significance. A high percentage of children with SAA and an unknown percentage of children with MDS respond to immunosuppressive therapy. Although immunosuppressive therapy may not, in itself, be harmful to children with MDS, it is not curative and does present some risk, including allergic reactions, infectious risks, and a delay in other potential therapies. In addition, children with HLA-matched related donors undergoing transplantation typically receive preparative regimens, which, though adequate for SAA, may not be adequate for MDS. Conversely, if an alternate donor stem cell transplant is performed for SAA or MDS, the morbidity and mortality are significant. Thus, alternate donor transplants are reserved for children with SAA only if unresponsive to immunosuppressive medications. Therefore, an accurate diagnosis is critically important in guiding the treatment of these children.

### Myelodysplastic Syndrome in Children with Down Syndrome

Children with Down syndrome have an increased probability of developing MDS and acute leukemia. Based on morphologic examination, it is extremely difficult, and unnecessary, to differentiate MDS from AML in children with Down syndrome. Therefore, these entities are generally considered together.

#### *Incidence and Epidemiology*

The true incidence of MDS and acute leukemia in children with Down syndrome is unknown. Some studies suggest that the increased risk of MDS and acute leukemia risk to be in the order of ten- to 20-fold. [232,233 and 234] Although older reports suggested that acute lymphoblastic leukemia (ALL) was the predominant form of acute leukemia in these children,[232,233 and 234] the frequency of MDS and AML is higher than previously thought, as improved methods to better identify megakaryocytic leukemias have been developed. Before the use of flow cytometry, megakaryocytic leukemias were easily confused morphologically, with ALL blasts having L2 morphology. Given these advances in diagnosis, there is consensus that the ratio of ALL to AML in children with Down syndrome is 1:1. [234]

It has been estimated that children with Down syndrome have a 400-fold increased probability of developing megakaryocytic leukemia (FAB M7), the most common form of AML in children with Down syndrome.[235,236] Large studies to better define the incidence and epidemiology of MDS and acute leukemia in children with Down syndrome are currently under way.

### *Etiology and Pathophysiology*

The reasons for the increased risk of MDS and acute leukemia in children with Down syndrome is not known, but some data suggest abnormalities related to chromosome 21 may be, at least in part, responsible for the increased risk of leukemogenesis. Genes that may have a causative role that are located on chromosome 21 include genes for superoxide dismutase, cystathionine-b-synthetase, and carbonyl reductase. In addition, many of the phenotypic features of Down syndrome have been mapped to chromosome 21, band q21.1-22.1, also site of the *AML-1* gene, a gene commonly involved in numerous AML-associated translocations [e.g., t(8;21)].[237,238,239,240,241 and 242] It is anticipated that information derived from the DNA sequencing of human chromosome 21 will help to identify other genes that may contribute to the development of leukemia in these children.[243] Taken together, available data have led to the postulate that children with Down syndrome are at an increased risk of developing MDS and acute leukemia as a result of developmental errors that disrupt hematopoiesis, ineffective regulation of granulopoiesis, immune deficiencies that lead to impaired immune surveillance, abnormal cell cycle kinetics, susceptibility to viral infections, a genetic predisposition to nondisjunction, increased chromosome fragility, impaired DNA repair mechanisms, and oncogene activation.[232]

### *Treatment and Outcome*

It has been demonstrated in several studies that children with Down syndrome, MDS, and AML can be cured with chemotherapy of moderate intensity.[240,244,245,246 and 247] In the largest of these studies, 118 children with Down syndrome and MDS or AML (Table 22-6) were prospectively treated with either intensively timed chemotherapy or conventional, standard-timed AML chemotherapy with or without subsequent matched related donor bone marrow transplantation.[240] Patients receiving intensively timed chemotherapy and those undergoing bone marrow transplantation had increased mortality as a result of excessive toxicity. Patients receiving standard-timing chemotherapy had a 4-year DFS of 88%, compared to 42% ($p$ <.001) for similarly treated patients with de novo AML who did not have Down syndrome (Fig. 22-4). This is exactly opposite to the outcomes of normal children who develop AML.[248] Based on the exceptionally good outcomes after only moderately intensive chemotherapy, investigators have postulated that MDS and AML are different or the host is different, or both, in children with Down syndrome.[240]

| | | DS | | No DS | |
|---|---|---|---|---|---|
| | n | 118 | n | 1,088 | p Value |
| Gender (M:F) | | 0.76 | | 1.18 | .058 |
| Race (white:other) | | 1.29 | | 1.85 | .138 |
| Age (y or [mean] interquartile range) | | 1.8 (1.3–2.4) | | 7.5 (2.5–13.2) | <.001 |
| Hemoglobin (g/dL [median] interquartile range) | | 8.1 (5.7–9.9) | | 8.2 (6.5–9.8) | |
| White blood cell count × 10³/μL interquartile range | | 7.5 (4.5–14.1) | | 19.9 (6.4–61.8) | <.001 |
| Platelets × 10³/μL interquartile range | | 29.0 (15–51) | | 52.0 (26–110) | <.001 |
| History of MDS | 23 | (20%) | 89 | (8%) | <.001 |
| FAB* | | | | | |
| MDS | 8 | (8%) | 39 | (4%) | .11 |
| FAB M0 | 10 | (10%) | 27 | (3%) | <.01 |
| FAB M1/2 | 10 | (10%) | 420 | (41%) | <.001 |
| FAB M3 | 2 | (2%) | 20 | (2%) | .58 |
| FAB M4/5 | 7 | (7%) | 383 | (37%) | <.0001 |
| FAB M6 | 3 | (3%) | 22 | (2%) | .853 |
| FAB M7 | 65 | (62%) | 96 | (9%) | <.001 |

FAB, French American British Cooperative Group.
*FAB M0 includes only those who had not transformed to acute myelogenous leukemia. M0 was included in Children's Cancer Group Study-2891 only in a 1:1 for DS and M6 for others. Ninety-four percent of cases were classified by FAB criteria.
From Lange BJ, Kobrinsky N, Barnard DR, et al. Distinctive demography, biology, and outcomes of acute myeloid leukemia and myelodysplastic syndrome in children with Down syndrome: Children's Cancer Group Studies 2861 and 2891. Blood 1998;91:608–615, with permission.

**TABLE 22-6. PRESENTATION OF ACUTE MYELOID LEUKEMIA OR MYELODYSPLASTIC SYNDROME (MDS) IN CHILDREN WITH AND WITHOUT DOWN SYNDROME (DS)**

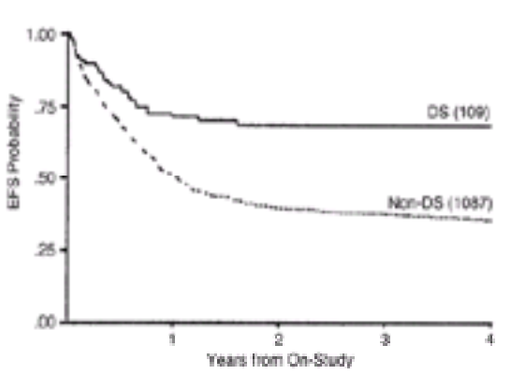


**FIGURE 22-4.** Kaplan-Meier plot of actuarial event-free survival (EFS) in children with and without Down syndrome (DS) in Children's Cancer Group studies 2861 and 2891. Numbers in parentheses indicate number at risk. EFS in DS is 68% (95% confidence interval, 47% to 84%); in non-DS, 35% (95% confidence interval, 30% to 41%). (From Lange BJ, Kobrinsky N, Barnard DR, et al. Distinctive demography, biology, and outcome of acute myeloid leukemia and myelodysplastic syndrome in children with Down syndrome: Children's Cancer Group Studies 2861 and 2891. Blood 1998;91:608–615, with permission.)

Potential differences in MDS and AML of Down syndrome and these diseases in children without Down syndrome are not readily apparent.[240] Investigations are focusing on the obvious differences in morphology (e.g., high predominance of megakaryocytic differentiation in Down syndrome), karyotype (e.g., an extra copy of chromosome 21), and immunophenotype between these different populations of patients. Differences in the host have included investigations of altered metabolism of cytarabine[240,249] and other drugs in children with Down syndrome. Despite these investigations, it is still not clear why children with MDS and AML have extremely good outcomes, but also are at greater risk of toxicity from therapy than children without Down syndrome.

### Therapy-Related Myelodysplastic Syndrome

Children and adults who have received ionizing radiation, cytotoxic chemotherapy (or both), particularly with alkylating chemotherapy and topoisomerase II inhibitors, are at an increased probability of developing therapy-related MDS. Given the different etiology, clinical characteristics, poor response to therapy, and generally poor prognosis, these disorders should be considered separately from de novo MDS.

Alkylating agents used to treat children and adults with Hodgkin's disease[250,251 and 252] and non-Hodgkin's lymphoma[252] are most commonly associated with the development of therapy-related MDS,[253] but MDS after the treatment of other diseases has also been reported.[254,255 and 256] Treatment-related MDS after exposure to alkylating agents develops approximately 5 years after exposure to the inciting agent. Cytogenetic abnormalities in chromosomes 5 and 7 are commonly seen.[257,258] In contrast, exposure to topoisomerase inhibitors, such as the epipodophyllotoxins (e.g., etoposide), are associated with abnormalities in chromosome band 11q23[258,259 and 260] and, hence, more AML and less MDS.

Nontransplant approaches to the treatment of patients with therapy-related MDS have been limited.[261] Hematopoietic stem cell transplantation has been used, but results, even in adults, are difficult to identify because most series report patients with therapy-related MDS among results for patients with de novo MDS, SAA, and AML. However, a review of published studies[175,177,181,183,184,185,186,187 and 188,262,263,264,265,266,267,268,269 and 270] suggests a very high probability of NRM, high probability of relapse, and a small, but not insignificant, chance of DFS. As seen in Figure 22-5, a transplant study of children with therapy-related MDS demonstrated a DFS probability at 2 years of 24%, with relapse and NRM both accounting for the majority of deaths.[267] However, overall survival for children with therapy-related MDS may be less than this as many children may not survive long enough to receive a transplant procedure.

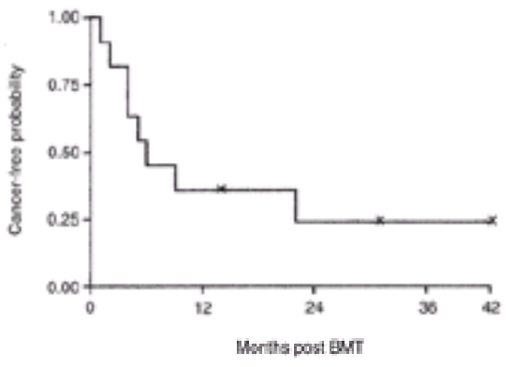


**FIGURE 22-5.** Kaplan-Meier plot of cancer-free survival after allogeneic bone marrow transplant (BMT) for therapy-related leukemia. Censored patients are shown by a cross. (From Leahey AM, Friedman DL, Bunin NJ. Bone marrow transplantation in pediatric patients with therapy-related myelodysplasia and leukemia. Bone Marrow Transplant 1999;23:21–25, with permission.)

As a result of dose escalation regimens and autologous transplantation for an increasing number of malignant [251,271,272] and nonmalignant disorders, there is general concern that an increase in the incidence in therapy-related MDS will be seen in the next decade.

## MYELOPROLIFERATIVE DISORDERS

### Transient Myeloproliferative Disorder in Children with Down Syndrome

#### *Incidence and Epidemiology*

Transient MPS disorder (TMD) is typically seen in the first several months after birth in children with Down syndrome. Although the true incidence of TMD is unknown, the incidence of severe TMD has been estimated to be as high as 10% for children with Down syndrome. [273,274] There are no published epidemiology studies of TMD in children with Down syndrome.

#### *Etiology and Pathogenesis*

TMD is characterized by an uncontrolled proliferation of myeloblasts. This disorder is differentiated from congenital AML by its tendency to resolve spontaneously within the first 3 months of life (range, birth to 7 months). [274,275,276,277,278,279] and [280] Differentiating between TMD and congenital AML can be difficult. Little data exist comparing TMD to congenital AML. As demonstrated in Table 22-7, children with TMD have a lower percentage of blasts in their bone marrow than in their peripheral blood. As a general pattern, TMD patients also have no cytogenetic abnormalities in their blasts, whereas AML patients had clonal cytogenetic abnormalities identified.

| Feature | TMD (n = 15) | AML (n = 13) |
|---|---|---|
| Age | 4 (0–34) days | 21 (6–30) months |
| Peripheral white blood cell count (× 1,000) | 72.9 (26.8–248.6) | 10 (1.8–40.6) |
| Peripheral blasts (%) | 57 (20–89) | 30 (0–68) |
| Hemoglobin | 17.1 (14.5–19.9) | 9.3 (3.6–14.9) |
| Platelets (× 1,000) | 137 (29–853) | 38 (10–56) |

From Hayashi Y, Eguchi M, Sugita K, et al. Cytogenetic findings and clinical features in acute leukemia and transient myeloproliferative disorder in Down syndrome. Blood 1988;72:15–23, with permission.

**TABLE 22-7. COMPARISON OF PRESENTING CHARACTERISTICS OF CHILDREN WITH DOWN SYNDROME AND TRANSIENT MYELOPROLIFERATIVE DISORDER (TMD) OR ACUTE LEUKEMIA (AML)**

Like AML, the myeloblasts in TMD are clonally derived [281] and frequently are of megakaryocytic origin with varying degrees of differentiation. [273,275]

#### *Clinical Presentation*

Because no population-based studies have yet been performed on TMD, information regarding the presentation and natural history of this disorder are based on several small series of patients. TMD typically presents in the first 3 months of life. Children may present at birth with hydrops fetalis secondary to anemia or cardiac dysfunction secondary to infiltration of cardiac muscle with myeloblasts. Other presenting signs and symptoms include organ infiltration with resulting hepatosplenomegaly; pleural, pericardial and peritoneal effusions; disseminated intravascular coagulopathy; renal failure; and respiratory failure. The complete blood cell count typically demonstrates an elevated white blood cell count with circulating myeloblasts.

Children with Down syndrome and TMD are known to develop AML and ALL. [282] Although the true incidence of progression to acute leukemia is unknown, one study found that 9 of 43 (21%) of children with Down syndrome and AML had a preceding history of TMD. [282]

#### *Treatment and Outcome*

The treatment for Down syndrome patients with TMD is generally supportive. Unless there is significant organ dysfunction, children with TMD can be followed closely without medical intervention. If significant organ dysfunction is present, therapeutic approaches include exchange transfusion, leukapheresis, and chemotherapy of varying intensities, with cytosine arabinoside most often used.

Although TMD commonly undergoes a spontaneous remission, it is not a benign disorder. Children with TMD can die as a result of hydrops fetalis, organ infiltration, renal failure, hepatic failure, respiratory failure, disseminated intravascular coagulopathy, and progression to AML or ALL. [275,283] The true mortality rate, however, is not currently known.

### Juvenile Myelomonocytic Leukemia

JMML is a rare, clonal abnormality of the pluripotent stem cell that is manifest as an MPS disease of early childhood. Besides the adult form of Ph $^{+}$ CML, JMML constitutes the most common form of MPS in childhood. As illustrated in Figure 22-1, JMML is probably a “bridging” disorder between the MPS and MDS diseases.

#### *Nomenclature and Diagnosis*

JMML represents a common name now generally accepted for this MPS disorder that has been referred to by many other names. JMML has been variously called *juvenile granulocytic leukemia*,[47,48] *infantile monosomy 7 syndrome*,[39,42,284,285] *chronic* and *subacute myelomonocytic leukemia*,[40] and the more commonly used term,

*juvenile CML* (JCML).[45,46] Because of the similarity of the name *JCML* to $Ph^+$ CML, many clinicians inappropriately considered JMML to be the childhood form of adult $Ph^+$ CML. Despite a similarity in the names, JMML and the adult form of CML have few clinical or biologic similarities. Confusion has also resulted because of apparent similarities in the characteristics for JMML and the adult form of MPS, CMML. These disorders have now been demonstrated to have some distinct differences, including differences in age of presentation, in the *in vitro* growth characteristics,[286] a lack of elevated fetal hemoglobin in CMML, and more complex cytogenetic characteristics in CMML. Given the confusion in nomenclature and similarities to other diseases, defining the clinical and laboratory characteristics of JMML has been challenging. Until recently, there were no widely accepted nomenclature or clinical and laboratory criteria for JMML.

An international consensus panel consisting of the JMML Working Group and the EWOG-MDS met in 1994 [11,43,287] and agreed on common clinical and laboratory criteria to diagnose JMML. The widespread acceptance of the term *JMML* and the international diagnostic criteria [11,43,287] (Table 22-8) have helped to decrease the significant confusion that previously existed for this disorder.

| |
|---|
| Required laboratory criteria (all three required) |
| No Philadelphia chromosome, or no *bcr/abl* rearrangement |
| Peripheral blood monocyte count >1 × $10^9$/L |
| Bone marrow blasts <20% |
| Suggestive clinical features |
| Hepatomegaly |
| Lymphadenopathy |
| Pallor |
| Fever |
| Skin rash |
| Additional criteria (minimum of two required) |
| Increased fetal hemoglobin (age corrected) |
| Myeloid precursors in peripheral blood |
| White blood cell count >10 × $10^9$/L |
| Clonal abnormalities, including monosomy 7 |
| Granulocyte-macrophage colony-stimulating factor hypersensitivity of myeloid progenitors in vitro |

**TABLE 22-8. DIAGNOSTIC CRITERIA FOR JUVENILE MYELOMONOCYTIC LEUKEMIA**

### *Biology*

Although early clonality studies suggested that JMML arose at the level of at least an immature myeloid precursor cell, [11,288,289,290,291] and [292] recent data suggest that JMML may arise in a pluripotent stem cell with involvement of the myeloid, erythroid, and megakaryocyte lineages as well as B lymphocytes and T lymphocytes.[11,293,294] This finding is further supported by the observation that JMML cells maintained in long-term culture-initiating cell assays maintain clonality. [11,295] This is in contrast to $Ph^+$ CML, also a disease initiating in a pluripotent hematopoietic stem cell, which becomes polyclonal in long-term culture-initiating cell assays.

Several potential causative mutations in JMML cells have been identified, including loss of heterozygosity of *NF1*[296] and activating *ras* mutations.[297,298] and [299] Approximately 15% of patients with JMML have clinically evident neurofibromatosis. [11,43,296,300,301] Abnormalities in *NF1* are also detectable in an additional 15% of JMML patients who do not have clinical manifestations of neurofibromatosis. [11,302] Activating point mutations in *RAS* genes have also been detected in 15% to 30% of JMML patient samples.[11,297,302,303] These groups of JMML patients with abnormalities in *NF1* and *RAS* (most commonly *N-RAS* and*K-RAS*) appear to be mutually exclusive.[302,303] The RAS family of proteins are of significance in JMML in that they are involved in signaling pathways for GM-CSF. [11,287,294,304] This deregulation in signaling results in the well-described pattern of GM-CSF hypersensitivity in *in vitro* dose response assays.[286] However, JMML cells do not show selective hypersensitivity to IL-3 despite similar signaling components to those of GM-CSF. [286] It has also been shown that *NF1* encodes for the protein neurofibromin, a protein that functions as a guanosine triphosphatase–activating protein, which inactivates Ras from its active guanosine triphosphate–bound state. [11,305,306] and [307] Therefore, *NF1* may act as a tumor suppressor gene in immature hematopoietic cells by negatively regulating Ras. [11,296,301]

A potential mouse model of JMML has recently been developed.[11,298,299] Mice that have homozygously deleted *Nf1* (termed $Nf1^{-/-}$) have an embryonic lethal defect that prevents development beyond day 13 to 14 of gestation. However, when hematopoietic stem cells present in the embryonic liver of the $Nf1^{-/-}$ mice are grown in *in vitro* colony assays, they demonstrate a pattern of GM-CSF hypersensitivity similar to that seen with JMML cells. In addition, when these cells are transplanted into irradiated recipients, the recipient mice develop an MPS disorder similar to that seen in children with JMML. [11] These data suggest that deregulated Ras signaling is a molecular hallmark of JMML, evident in 50% to 60% of JMML patients.[11] The molecular abnormalities occurring in the remaining patients have yet to be determined.

### *Epidemiology*

The true incidence of this disorder has not yet been established. Patients with JMML have a male predominance, and, though occasionally presenting at ages older than 5 years of age, it is most common in children younger than 5 years. As a result of the rarity of the disease and the absence of uniform registration of patients, epidemiologic studies to address potential environmental and genetic factors have not yet been possible.

### *Clinical Presentation*

As is indicated in Table 22-8, children with JMML usually present with signs and symptoms indicative of a heavy tumor burden of organ-infiltrating cells. Hence, hepatomegaly, splenomegaly, generalized lymphadenopathy, and skin rash are commonly seen. Due to a relatively high association with neurofibromatosis, patients may also have neurofibromas and café-au-lait spots. Laboratory abnormalities include an elevated white blood cell count with monocytosis, anemia, thrombocytopenia, elevated fetal hemoglobin, and hypergammaglobulinemia. JMML may transform to a blastic phase, but this is unusual, occurring in less than 20% of patients.[39] Patients die as a result of resistant disease with organ infiltration (i.e., skin, lungs, liver, spleen, and intestines), which results in organ dysfunction, infection, and bleeding.

### *Treatment*

JMML has a very heterogeneous clinical course with at least three patterns. Many patients die within a year of diagnosis with a rapidly progressive course while a second group may have a period of treatment-responsive disease with intensive, AML-like therapy, followed by progressive disease. [40,309] Rare patients, however, have long-term survival with spontaneous remissions. Although age, platelet count, and HbF at diagnosis may be predictive for the length of survival in patients not undergoing transplantation,[43] the biologic parameters that identify patients with differing clinical courses and response to therapy have not been clearly identified.

Although the potential value of intensive, AML-like chemotherapy for patients with JMML has been suggested based on superior survival for intensively treated patients in comparison to those patients who received less intensive therapy, [43,309] many JMML patients fail to enter a remission with chemotherapy. For those patients who do achieve a remission after intensive therapy, these remissions tend to be of a short duration. [43,126,165,310,311] To gather more information about the role of AML-like chemotherapy for the induction of remission in JMML, the Children's Cancer Group has prospectively enrolled JMML patients in recent AML trials using intensively timed combination chemotherapy.[168] Preliminary results from the Children's Cancer Group demonstrate that some JMML patients respond initially to aggressive chemotherapy, and that results after aggressive chemotherapy may be better than limited therapy.

Lower dose chemotherapy regimens (e.g., cytosine arabinoside) has been used with some responses noted. [312,313] However, the overall efficacy of this therapeutic approach is currently unknown. Similarly, interferon-a, while demonstrating inhibitory effects on spontaneous cell growth *in vitro*, has shown little efficacy in the treatment of JMML.[314]

Isotretinoin (13-*cis*-retinoic acid), like interferon-a, also has an inhibitory effect on spontaneous growth *in vitro*, but unlike interferon-a, may induce remission in at least a portion of JMML patients.[308] The efficacy of isotretinoin in a portion of JMML patients has been suggested in a phase II clinical trial. [308] Despite this preliminary

success, it is clear that therapy with isotretinoin is not curative.

Newer agents, including the farnesyltransferase inhibitors (FTIs),[294,295,315] novel retinoids,[316] GM-CSF antagonists,[317,318] and GM-CSF/diphtheria immunotoxins,[319] are also being developed. These new agents specifically target mechanisms thought to be operative in JMML. The new class of agents targeted to the Ras pathway, the FTIs, are particularly interesting. For Ras proteins to serve as a "master switch" for signal transduction, they must be bound to the inner surface of the plasma membrane. To do this, they must undergo a series of posttranslational modifications, the first of which is the addition of a 15-carbon isopropenyl group to Ras.[315,320,321,322,323 and 324] This step is catalyzed by the enzyme farnesyl-protein transferase. A number of inhibitors of this enzyme (e.g., FTIs) have been developed that demonstrate antitumor effects in various *in vitro* and xenogeneic mouse models.[325,326,327,328,329,330,331,332,333 and 334] Some of these agents have shown efficacy in inhibiting JMML cell growth *in vitro* and in *NF1*-deficient cells.[294,335,336] The utility of these agents in the treatment of children with JMML is not yet known, but will soon be studied in prospective clinical trials.

Allogeneic hematopoietic stem cell transplantation continues to offer the greatest probability of long-term DFS.[43,180,337,338,339,340 and 341] However, despite the curative potential of allogeneic transplantation, frequent and early relapse of JMML is problematic, with survival in 20% to 30% of patients.[43,180,337,338,339 and 340,342] Although allogeneic stem cell transplantation is potentially curative in a minority of patients, a number of issues involving transplantation remain controversial.[341] These unresolved issues include

1. The preferred stem cell source if an HLA-matched related donor is not available
2. The safety and efficacy of intensive chemotherapy before transplantation
3. The role of splenectomy pre-transplant and inclusion of TBI as part of the conditioning regimen
4. The role of T-cell depletion
5. The strength of the graft-versus-leukemia reaction in JMML

Children with JMML should be enrolled in prospective clinical trials currently in progress in Europe (EWOG-MDS), in North America (COG), and elsewhere.

### Other Myeloproliferative Disorders of Childhood

$Ph^+$ CML and JMML represent the vast majority of the MPS disorders of childhood. There are, however, rare, anecdotal reports of other MPS diseases in children that are far more common in adults.[11] These other disorders include polycythemia vera,[11] and primary familial and congenital polycythemia (PFCP).[343,344] Although traditionally classified as MPS disorders, essential thrombocythemia and AMM may be more similar to the MDS syndromes or represent "bridging" disorders ( Figure 22-1).[32,345,346 and 347]

#### *Polycythemia Disorders*

Polycythemia in a child may be the result of primary polycythemia due to polycythemia vera or PFCP, secondary polycythemia resulting from multiple causes, including abnormal hemoglobins and excessive erythropoietin, or the relative polycythemias that are the result of decreased plasma volume.

Polycythemia vera, rarely reported in children, is a clonal disorder of hematopoietic stem cells that may effect the erythroid, myeloid, and megakaryocytic lineages. It may progress to a more rapidly progressive MPS disorder or to AML. It has been suggested that polycythemia vera may be associated with a hypersensitivity of stem cells to insulin-like growth factor-1.[348] Children, like adults, may present with signs and symptoms associated with the increased red cell mass such as headache, dizziness, fatigue, night sweats, and pruritus. As a result of the increased red cell mass, patients may have hyperviscosity of the blood that can result in thrombosis and central nervous system ischemia. Physical examination may be remarkable for facial redness and hepatosplenomegaly. Laboratory evaluation demonstrates an elevated hematocrit. A moderate leukocytosis and thrombocytosis are also commonly present. The same criteria for a diagnosis of polycythemia vera, commonly used in adults, should be used in children.[349]

Numerous approaches, with variable safety and efficacy, have been used in the treatment of polycythemia vera in adults. These include phlebotomy, busulfan, chlorambucil, $^{32}P$,[350,351] hydroyurea,[352,353] and interferon-a.[354] Aspirin[355] and anagrelide[11] have been used in attempts to control thrombotic complications. Aspirin has not been shown to be useful, whereas the efficacy of anagrelide is unknown. As a result of the long survival of adult patients with polycythemia vera,[356,357] allogeneic hematopoietic stem cell transplantation has only rarely been used in the treatment of these patients.[358,359,360 and 361] Of nine reported transplants, five patients are reported to be long-term, disease-free survivors. Consideration of stem cell transplantation is generally given only to patients with a history of significant thrombotic events or polycythemia vera that has progressed to AML.

PFCPs are not true MPS disorders. They are inherited in an autosomal dominant fashion. Although this disorder is generally benign without the need for therapy, thrombotic events have been reported.

#### *Essential Thrombocythemia*

Essential thrombocythemia is extremely rare in children. It is characterized by an isolated increase in the megakaryocytic lineage with abnormal platelet function. However, an elevated platelet count in children is far more common as a result of other MPS disorders, nutritional causes, or as an acute phase reaction secondary to infection or inflammation. Adult patients with essential thrombocythemia may present with thrombotic episodes or splenomegaly. Diagnostic criteria for essential thrombocythemia have been proposed.[362] Adult patients have a median survival of at least 10 years,[355,357] with most deaths secondary to thrombosis. Progression to AML has been noted in some patients, but usually only those exposed to chemotherapy agents. Although it is generally recommended that young, asymptomatic patients not be treated, treatment has been attempted with hydroxyurea, interferon-a, and anagrelide.[11] Successful hematopoietic stem cell transplantation has been reported in three adult patients.[361]

#### *Agnogenic Myeloid Metaplasia/Primary Myelofibrosis*

Although rare, AMM has been reported in children.[347] This "bridging" disorder (Fig. 22-1) is characterized by marrow fibrosis, splenomegaly, anemia, and extramedullary hematopoiesis. It has been suggested that AMM in children may in fact be FAB M7 AML. Patients typically present with signs and symptoms resulting from splenomegaly and anemia. In children, AMM should be differentiated from metastatic neoplasms, connective tissue disease, and metabolic and bone diseases. Survival in adults is variable, ranging from 1 to 30 years.[357,363,364] A prognostic scoring system has been proposed.[364] Numerous treatment approaches have been attempted with variable degrees of success. These include the use of splenectomy,[363,365,366] splenic irradiation, transfusions, androgens, corticosteroids, hydroxyurea, and interferon-a. Hematopoietic stem cell transplantation has been successfully used to treat AMM, with a total of 29 patients reported.[347,361,367,368,369,370,371,372 and 373] After transplantation, surviving patients with donor engraftment had the resolution of myelofibrosis. It is therefore generally recommended that stem cell transplantation be considered in young patients with poor prognostic scores.[364]

## FUTURE DIRECTIONS

Given the rarity of the MDS and MPS disorders, the success of future treatment approaches is critically dependent on a unified approach to therapy. As the European community (EWOG-MDS), North America (COG), and others embark on novel, prospective clinical trials that will likely capture the majority of children with these disorders in these geographic regions, it is anticipated that new knowledge and improvements in treatment will be forthcoming.

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# HODGKIN'S DISEASE

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## INTRODUCTION

The original paper by Hodgkin in 1832 was entitled “On Some Morbid Appearances of the Absorbent Glands and Spleen.” [1] In that era of anatomic description of disease, investigators were concerned with differentiating inflammatory disease from infection or idiopathic hypertrophy of the lymphoid organs. Not until the second half of the nineteenth century, as the criteria for making diagnoses came to depend more on microscopic morphology, did investigators recognize that abnormal giant cells were present in Hodgkin's material. Sternberg in 1898 [2] and Reed in 1902 [3] are generally credited with the first definitive and thorough descriptions of the histopathology of Hodgkin's disease. Reed in particular gave a precise description of the multinucleated giant cells in this disease, which led her finally to refute the idea that it was an unusual form of tuberculosis despite the frequent association of the two diseases in the same patient. After the histologic definition of the disease was established, Fox, [4] in 1926, reexamined the histologic features of Hodgkin's original seven patients and concluded that three of them, one of whom was a pediatric patient, met the new criteria for definition of the disease.

In the ensuing years, although Hodgkin's disease was recognized as a possible malignancy, the potential of an infectious or autoimmune etiology was still considered. [5] The pleomorphic nature of the cellular infiltrate in Hodgkin's disease made investigators uncomfortable with the idea that this was a clonal proliferation of a single malignant cell. However, the successful cultivation of Reed-Sternberg cells [6] permitted the demonstration of the cells' malignant nature and reinforced the idea that Hodgkin's disease was truly a malignant disorder.

Initial attempts with radiotherapy for this disease were disappointing; dramatic regression was followed by recurrence and, inevitably, death. [7] Improvements in radiation therapy technology eventually resulted in the cure of early stage disease with radiotherapy alone. In 1940, as a by-product of wartime work on compounds related to the mustard gases, nitrogen mustard's powerful lymphocytolytic effects were discovered. [8] Experimental studies indicating the advantage of using combinations of non–cross-resistant antineoplastic agents with nonoverlapping toxicities led to the introduction in 1964 of the four-drug MOPP regimen [mechlorethamine (nitrogen mustard), Oncovin (vincristine), procarbazine, prednisone]. [9] MOPP chemotherapy was the first effective systemic therapy for Hodgkin's disease. Trials using MOPP chemotherapy in adult patients produced prolonged disease-free survival in approximately 50% of patients when MOPP was administered at full doses. [10] Subsequently, pediatric trials demonstrated similar or better outcomes after MOPP chemotherapy. [11,12,13,14 and 15] With improved survival after MOPP, investigators appreciated that both adults and children were vulnerable to its adverse effects, which consist of an increased risk of acute myelogenous leukemia (AML) and infertility. The development of the non–cross-resistant ABVD regimen [Adriamycin (doxorubicin), bleomycin, vinblastine, dacarbazine] in the 1970s provided effective systemic therapy for Hodgkin's disease that was not associated with an excess risk of secondary AML or infertility. [16] ABVD was initially used in adult trials to salvage patients who did not respond to MOPP chemotherapy. [17] The lack of leukemogenesis and permanent gonadal toxicity and superior treatment outcomes has led to the standard use of ABVD in adults with newly diagnosed Hodgkin's disease. However, the sole use of ABVD therapy in children has been less popular because of concerns about potential cardiopulmonary toxicity.

With greater appreciation of treatment sequelae after standard-dose radiotherapy and non–cross-resistant chemotherapy, pediatric investigators modified treatment strategies in the 1980s to address the specific needs of children. Combined-modality therapy regimens evolved in which cycles of chemotherapy replaced a portion of the radiation therapy in laparotomy-staged children with Hodgkin's disease. [11,18,19] The success of this approach coupled with advances in diagnostic imaging technology ultimately resulted in the abandonment of surgical staging in the 1990s. This decade also saw the evolution of risk-adapted trials in which patients with favorable clinical presentations received combined-modality treatment prescribing fewer cycles of multi-agent chemotherapy and lower radiation doses and treatment volumes. [20,21,22,23 and 24] Currently under investigation are novel approaches using compacted dose-intensive multi-agent chemotherapy for patients with advanced and unfavorable disease.

## EPIDEMIOLOGY

Hodgkin's disease has a unique bimodal age distribution that differs geographically and ethnically. In industrialized countries, the early peak occurs in the middle to late 20s and the second peak after the age of 50 years. In developing countries, the early peak occurs before adolescence. Epidemiologic studies demonstrate three distinct forms of Hodgkin's disease: a childhood form (in patients aged 14 years or younger), a young adult form (in patients aged 15 to 34 years), and an older adult form (in patients aged 55 to 74 years). [25] There is also a slight overall male predominance in the incidence of Hodgkin's disease, which is most marked in the childhood form. [26] In adolescents, the incidence between males and females is roughly equal, and most older adolescent patients are white. Hodgkin's disease is rarely diagnosed in children younger than 5 years.

The childhood form of Hodgkin's disease tends to increase with increasing family size and decreasing socioeconomic status. In contrast, the young adult form of Hodgkin's disease is associated with a higher socioeconomic status in industrialized countries. The risk for young adult Hodgkin's disease decreases significantly with increased sibship size and birth order. [27] Histologic subtypes also show variability related to age at diagnosis. Mixed cellularity (MC) Hodgkin's disease is more common at younger ages, whereas nodular sclerosing Hodgkin's disease has a higher incidence in more affluent societies.

### Epstein-Barr Virus and Hodgkin's Disease

The epidemiologic characteristics of Hodgkin's disease suggest that its etiology may vary by age at presentation. [5,28] In the young adult form, delayed exposure to an infectious agent has been proposed as a risk factor for the development of Hodgkin's disease because its epidemiologic features are similar to that seen with paralytic poliomyelitis. Early and intense exposure to an infectious agent might increase the risk for the childhood form of Hodgkin's disease. Epstein-Barr virus (EBV) has been implicated in the causation of Hodgkin's disease by both epidemiologic and serologic studies. The large proportion of patients with Hodgkin's disease who have high EBV antibody titers suggests that enhanced activation of EBV may precede the development of Hodgkin's disease. This hypothesis is also supported by *in situ*

hybridization evidence of EBV genomes in Reed-Sternberg cells.[29] In cases associated with EBV, the virus is localize to the Reed-Sternberg cell, EBV latent gene products are expressed, and the EBV infection is clonal. EBV-associated antigens have been demonstrated in Hodgkin's tissues.[30] Both Reed-Sternberg cells and their variants consistently express Epstein-Barr nuclear antigen 1, but Epstein-Barr nuclear antigen 2, viral capsid antigen, early antigen, and membrane antigen have not been found.[30] EBV strain subtypes identified within Reed-Sternberg and Hodgkin's disease also vary geographically. EBV strain type 1 is predominant in the United Kingdom, South Africa, Australia, and Greece, whereas EBV type 2 is predominant in Egypt. The presence of infection by both EBV strains in 21% of cases supports the possibility of an underlying immune deficiency in these cases.

The incidence of EBV-associated Hodgkin's disease varies by age, sex, ethnicity, histological subtype, and regional economic level.[31,32] EBV-positive tumor genomes are more frequently observed in children aged 10 years or younger and in children living in developing countries. The incidence of EBV-associated Hodgkin's disease also varies by ethnic background, as evidenced by its presence in 93% of Asian, 86% of Hispanic, 46% of white, and 17% of African-American children with Hodgkin's disease in one series. EBV latent membrane protein (LMP) 1 expression varies among the histologic subtypes and is found in up to 96% of patients whose disease is characterized by MC, in 34% of patients with nodular sclerosing disease, and in 10% of those with lymphocyte predominance.

Clonality studies indicate EBV infection precedes expansion of the tumor cell population. Infected Hodgkin's and Reed-Sternberg (HRS) cells express high levels of LMP1, a viral protein that resembles a constitutively activated member of the tumor necrosis factor (TNF) receptor superfamily. LMP1 interacts with TNF receptor–associated factors that lead to activation of transcription factor NFkB and modulation of apoptotic and growth pathways.[33] LMP1 expression is also associated with up-regulation of cellular bcl2, interleukin-10 (IL-10), and major histocompatibility complex class I proteins in some but not all cell lines. These data have led to the speculation that EBV is directly involved in the pathogenesis of some cases of Hodgkin's disease. Whether EBV, either alone or with other carcinogens, plays a direct role in the pathogenesis of Hodgkin's disease is unknown. The disease may represent a common result of multiple pathologic processes that include viral infection and exposure of a genetically susceptible host to a sensitizing agent.

### Familial Hodgkin's Disease

Clustering of cases of Hodgkin's disease within families or races may suggest a genetic predisposition to the disease or a common exposure to an etiologic agent. Studies of affected families have suggested an increased association of Hodgkin's disease with specific HLA antigens.[34] The concordance of Hodgkin's disease in first-degree relatives (including siblings), particularly of the same gender, and in parent-child pairs has been noted in numerous reports.[31] In families in which twins are concordant, elevated risk of Hodgkin's disease ranges from threefold among first-degree relatives to sevenfold in siblings.[35]

Reports of Hodgkin's disease in both marriage partners are extremely rare, as are data suggestive of transplacental transmission. Hodgkin's disease is diagnosed more commonly in persons whose immune system is abnormal, a finding that may reflect the slight increase in familial incidence.[36] The etiologic factors underlying the immune deficiency include genetic (e.g., ataxia-telangiectasia), infectious (e.g., human immunodeficiency virus), and iatrogenic agents.[36]

## BIOLOGY

The HRS cells, lymphocytic and histiocytic (L&H) cells, and their variants compose the malignant cells of Hodgkin's disease. Until recently, their limited numbers in affected tissues hampered the elucidation of their nature and origin. Advances in the field of immunohistology and molecular biology have improved understanding regarding the origin and clonality of these atypical multinucleated giant cells. The presence of identical immunoglobulin gene rearrangements in HRS cells in 90% of cases and L&H cells in 100% of cases supports their origin from a single transformed B cell that undergoes subsequent monoclonal expansion.[37,38] Immunohistological studies demonstrate two distinct immunophenotypes of the malignant cells in Hodgkin's disease ( Table 23-1). Immunophenotype I is characterized by the consistent expression of CD20 and J chain, and absence of CD30 and CD15. Immunophenotype II is characterized by the consistent expression of CD30, frequent expression of CD15, and consistent absence of J chain. L&H cells have immunophenotype I, whereas other HRS cells have immunophenotype II, a variation that correlates well with the unique clinical features of localized lymphocyte predominance disease. This distinction forms the basis for the revised European-American classification of lymphoid neoplasms, which proposes to combine all histological subtypes with immunophenotype II, including nodular sclerosis (NS), MC, and lymphocyte depletion, under the designation of classical Hodgkin's disease.[38] These findings designate a clear distinction between lymphocyte predominance Hodgkin's disease and classical Hodgkin's disease. In this classification, the histologic subtype that is similar in growth and cellular composition to lymphocyte predominance, but has immunophenotype II characteristic of classical Hodgkin's disease, is designated as nodular lymphocyte–rich classical Hodgkin's disease.

| | Immunophenotype I: Lymphocytic predominance Hodgkin's disease | Immunophenotype II: Classical Hodgkin's disease |
|---|---|---|
| Antigen | | |
| J chain | +(-) | - |
| CD20 | + | -/+ |
| CD79a | + | -/+ |
| CD30 | - | + |
| CD15 | - | +/- |
| Cell type | | |
| Lymphocytic and histiocytic | + | - |
| Hodgkin and Reed-Sternberg | - | + |

Adapted from Stein H, Diehl V, Marafioti T, et al. The nature of Reed-Sternberg cells, lymphocytic and histiocytic cells and their molecular biology in Hodgkin's disease. In: Mauch PM, Armitage JO, Diehl V, Hoppe RT, Weiss LM, eds. Hodgkin's disease. Philadelphia: Lippincott Williams & Wilkins, 1999:121.

**TABLE 23-1. IMMUNOPHENOTYPES OF LYMPHOCYTIC AND HISTIOCYTIC CELL AND CLASSICAL HODGKIN AND REED-STERNBERG CELL**

Immunophenotypic evaluation of L&H cells of lymphocyte predominance Hodgkin's disease uniformly demonstrates the expression of B-cell antigens (CD19, CD20, CD22, CD79a), consistent with their derivation from B lymphocytes.[39,40] HRS cells of classical Hodgkin's disease usually lack lineage-specific antigens and express some unusual molecules, including those characteristic of dendritic antigen-presenting cells (restin, fascin, thymus, and activation-regulated chemokine).[39] Rearrangement and somatic hypermutation of the immunoglobulin heavy-chain variable genes suggest that Reed-Sternberg cells derive from germinal center B lymphocyte that carries nonproductive immunoglobulin genes and resists culling by apoptosis.[40] Recent studies have provided evidence that suggests that deregulation of the nuclear transcription factor NFkB in HRS cells may be a mechanism that prevents apoptosis. In 10% to 20% of cases of classical Hodgkin's disease, the antigen profile of HRS cells supports a derivation from activated T lymphocytes (CD3, CD4, CD8, T-cell receptor b chain).[40] Unequivocal evidence of T-cell–derived HRS, however (e.g., the presence of clonally rearranged T-cell receptor genes) has not been demonstrated.[41]Table 23-2 summarizes the surface phenotype of the histologic subtypes of Hodgkin's disease.

| | Histologic subtype | | | | |
|---|---|---|---|---|---|
| Antigen | Lymphocytic predominance | Nodular sclerosing | Mixed cellularity | Lymphocytic depletion | Anaplastic large cell lymphoma |
| LCA (CD45) | + | - | - | - | - |
| Ki-1 (CD30) | +/- | + | + | + | + |
| EMA/MI (CD15) | - | + | + | + | + |
| CBF (CD20) | + | +/- | +/- | +/- | +/- |

Adapted from Hsiaolin HC, Brubby AM, Cornelius MF. The cellular biology of the Reed-Sternberg cell. Blood 1996;84:1035.

**TABLE 23-2. IMMUNOPHENOTYPE OF HODGKIN'S DISEASE SUBTYPES AND ANAPLASTIC LARGE CELL LYMPHOMA**

Immunophenotyping of HRS cells has indicated expression of certain activation antigens, including the IL-2 receptor, Ki-1, the transferrin receptor, and HLA-DR epitopes. Initial reports that CD30 expression was restricted to HRS cells proved erroneous. The antigen was subsequently found to be expressed on activated or transformed cells, including T and B lymphocytes and macrophages, as well as in other lymphoproliferative disorders, including anaplastic large cell lymphoma. [42] Soluble CD30 levels in sera of patients with Hodgkin's disease have correlated with disease activity. [43] Table 23-2 contrasts the immunophenotype of the Hodgkin's histologic subtypes and anaplastic large cell lymphoma (see Chapter 24).

Hodgkin's disease is characterized by cytokine-producing and cytokine-responding cells. Many of the nonspecific clinical features in patients with Hodgkin's disease result from cytokine production by HRS cells and neighboring inflammatory cells. Among these are high levels of CD30 and CD40, members of the TNF receptor superfamily.[44] CD30, CD40, or EBV LMP1 can activate NFkB and c-*Jun* N-terminal kinase pathways, which regulate HRS cell proliferation, expression of adhesion molecules, and secretion of cytokines.[44] The unique histopathologic features, such as eosinophilia and collagen sclerosis, have been attributed to the production of cytokines such as IL-4, IL-5, eotaxin, IL-6, IL-7, TNF, lymphotoxin, transforming growth factor-b (TGF-b), and basic fibroblast growth factor. [44] Adhesion molecules regulated by cytokines are thought to influence the interaction of HRS cells with neighboring T lymphocytes and metastatic capacity of Hodgkin's disease. Systemic symptoms have been best correlated with elevated serum levels of IL-6 and immunosuppression in untreated patients with TGF-b. [44] Elevated serum levels of CD30 and CD25 have been associated with advanced stage, constitutional (B) symptoms, and poor outcome. [43] The unusual expression of CD30 and CD25 on HRS cells forms the basis of novel immunotherapies targeting tumor cells with lethal toxins or effector immune cells. [45,46] Table 23-3 summarizes the relationship among cytokine production and common clinical and pathologic features of Hodgkin's disease.

| Clinical and pathologic features of Hodgkin's disease | Cytokines |
|---|---|
| Constitutional ("B") symptoms | TNF, LT-α, IL-1, IL-6 |
| Polykaryon formation | Interferon-γ, IL-4 |
| Sclerosis | TGF-β, LIF, PDGF, IL-1, TNF |
| Acute phase reactions | IL-1, IL-6, IL-11, LIF |
| Eosinophilia | IL-5, granulocyte M-CSF, IL-2, IL-3 |
| Plasmacytosis | IL-6, IL-11 |
| Mild thrombocytosis | IL-6, IL-11, LIF |
| T-cell and Hodgkin's and Reed-Sternberg cell interaction | IL-1, IL-2, IL-6, IL-7, IL-9, TNF, LT-α, CD30L, CD40L, B7 ligands (CD80 and CD86) |
| Immune deficiency | TGF-β, IL-10 |
| Autocrine growth factors (?) | IL-6, IL-9, TNF, LT-α, CD30L, M-CSF |
| Increased alkaline phosphatase | M-CSF |
| Neutrophil accumulation/activation | IL-8, TNF, TGF-β |

IL, interleukin; LIF, leukemia inhibitory factor; LT, lymphotoxin; M-CSF, macrophage colony-stimulating factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor.
Adapted from Kadin ME, Lieberwitz DN. Cytokines and cytokine receptors in Hodgkin's disease. In: Mauch PM, Armitage JO, Diehl V, Hoppe RT, Weiss LM, eds. Hodgkin's disease. Philadelphia: Lippincott Williams & Wilkins, 1999:139.

**TABLE 23-3. CLINICAL AND PATHOLOGIC FEATURES OF HODGKIN'S DISEASE RELATED TO CYTOKINE PRODUCTION**

## PATHOLOGY

Hodgkin's disease is unique among the lymphomas because its malignant cells account for less than 1% of the total cell population of the tumor. The majority of the tumor is composed of an infiltrate of inflammatory cells (e.g., histiocytes, plasma cells, lymphocytes, eosinophils, neutrophils) and fibrosis, which develops as a result of cytokine release. The HRS cell (or its variant) and reactive cellular background are required to establish the diagnosis, because cells similar to the HRS cell may occur in other neoplastic and reactive processes, including lymphoid hyperplasia associated with infectious mononucleosis, non-Hodgkin's lymphomas (NHLs), and nonlymphoid malignant diseases, including carcinomas and sarcomas. The classic Reed-Sternberg cell is large (greater than or equal to 15 to 45 µm in diameter), with abundant cytoplasm and either multiple or multilobed nuclei. The nuclear membrane is usually intensely stained, and the delicate chromatin network within it typically gives way to a peculiar halo-like clear zone around the nucleolus that is often said to resemble an “owl's eye.” The nucleoli are also large and prominent ( Fig. 23-1).

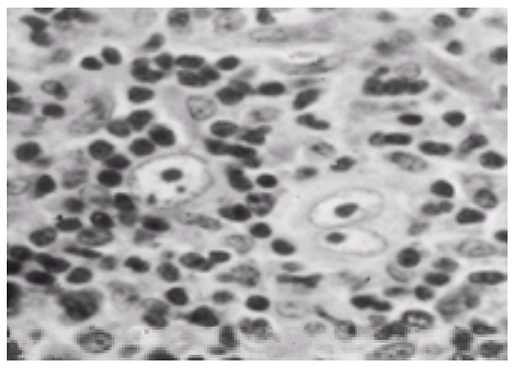


**FIGURE 23-1.** Characteristic Reed-Sternberg cell and mononuclear variant of Hodgkin's disease (hematoxylin and eosin; ×400).

Variants of the Reed-Sternberg cell include the lacunar cell, characteristically seen in nodular sclerosing disease. This variant appears as a cell in a space because of the artifactual retraction of the abundant pale cytoplasm after formalin fixation. Another variant, the frequently seen Hodgkin's cell, has the nuclear and nucleolar features of the Reed-Sternberg cell. In some patients, Hodgkin's cells are more pleomorphic and may be difficult to distinguish from cells present in diffuse pleomorphic histiocytic lymphoma.[47]

## DEFINITION OF HISTOLOGIC SUBTYPES

The most universally accepted histologic classification, the Rye classification system, defines four histologic subtypes of Hodgkin's disease: lymphocytic predominance (LP), MC, lymphocytic depletion (LD), and NS.[47] Historically, prognosis in the first three categories was linked to the ratio of lymphocytes to abnormal cells. Since the development of highly curative treatment regimens, however, all histologic subtypes of Hodgkin's disease are equally responsive to treatment.

In LP Hodgkin's disease, the lymph node architecture may be partially or completely destroyed. Because the cellular proliferation consists of benign-appearing lymphocytes and, sometimes, histiocytes, this subtype may be misinterpreted as reactive hyperplasia. Multiple tissue sections may need to be examined before a diagnostic Reed-Sternberg cell is identified; fibrosis usually is not seen. LP Hodgkin's disease affects 10% to 15% of patients, is more common among male and younger patients, and usually presents as clinically localized disease.

Reed-Sternberg cells and their variants are often numerous (5 to 15 per high-power field) in tissue samples of MC Hodgkin's disease. The lymph node is usually diffusely effaced and contains an inflammatory background of lymphocytes, plasma cells, eosinophils, histiocytes, and malignant reticular cells. Fine interstitial fibrosis may be seen, and focal necrosis may be present but usually is not marked. This subtype is observed in approximately 30% of patients, is more common in children aged 10 years or younger, and frequently presents as advanced disease with extranodal involvement. [48]

LD Hodgkin's disease is rare in children, but it is common in human immunodeficiency virus–infected patients. The presence of numerous, bizarre, malignant reticular cells, many Reed-Sternberg cells, and few lymphocytes characterizes this subtype. Diffuse fibrosis and necrosis are common. Clinical features of LD Hodgkin's include widespread disease that involves the bones and bone marrow. A significant proportion of cases previously designated LD Hodgkin's disease may actually represent diffuse large cell lymphoma.

Affecting approximately 40% of younger patients and 70% of adolescents, NS Hodgkin's disease is the most common subtype.[48] It is characterized by the lacunar variant of the Reed-Sternberg cell and, in most cases, a thickened lymph node capsule. Orderly collagenous bands emanate from the capsule and divide the lymphoid tissue into circumscribed nodules (Fig. 23-2). This process has a striking propensity to involve the lower cervical, supraclavicular, and mediastinal lymph nodes. Because of the abundance of collagen, the radiographic appearance of these lesions (particularly in the mediastinum) may only slowly return to normal, even when the patient is responding to therapy.

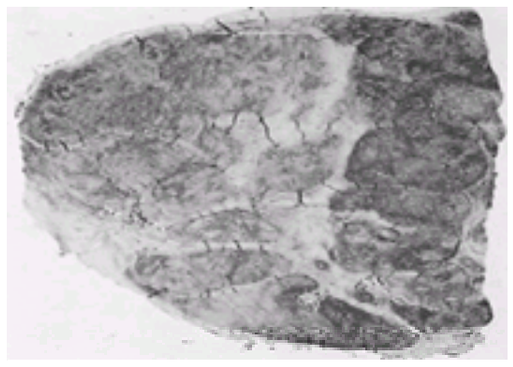


**FIGURE 23-2.** Lymph node, nodular sclerosing Hodgkin's disease. Cellular nodules are surrounded by dense fibrous bands (hematoxylin and eosin; ×8).

## CLINICAL PRESENTATION

### Lymphadenopathy

Usually patients present with painless supraclavicular or cervical adenopathy. Affected lymph nodes are firmer than inflammatory nodes, they feel rubbery, and they may be sensitive to palpation if they have grown rapidly. At least two-thirds of patients present with some degree of mediastinal involvement ( Fig. 23-3), which may cause a nonproductive cough or other symptoms of tracheal or bronchial compression. Posteroanterior and lateral thoracic radiographs should be performed as soon as Hodgkin's disease becomes part of the differential diagnosis. Airway patency should be fully assessed before the patients undergo any procedure requiring sedation. In younger children, mediastinal lymphadenopathy may be difficult to distinguish from a large, normal thymus. Infrequently, axillary or inguinal lymphadenopathy is the first presenting sign. Primary disease presenting in a subdiaphragmatic site is rare and occurs in only approximately 3% of cases. [49]

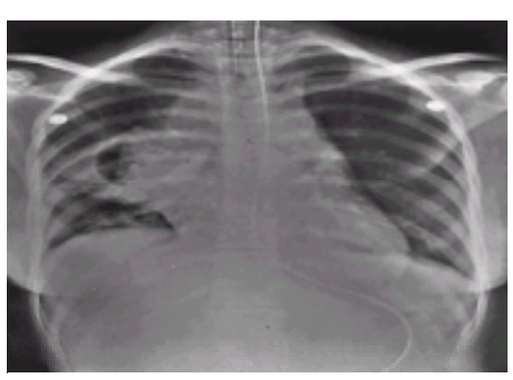


**FIGURE 23-3.** Anteroposterior chest film of a patient with significant tracheal compression below the distal endotracheal tube. Emergency radiotherapy may be required for such patients before staging procedures can be performed.

### Systemic Symptoms

Nonspecific systemic symptoms may include fatigue, anorexia, and slight weight loss. Three specific constitutional symptoms correlate with prognosis: unexplained fever with temperatures above 38.0°C orally, unexplained weight loss of 10% within 6 months preceding diagnosis, and drenching night sweats. Some studies suggest that night sweats are less prognostically important than other systemic symptoms.[50,51]

Pruritus is another systemic symptom commonly observed in patients with Hodgkin's disease, which lacks the prognostic significance associated with fever, sweats, and weight loss. Pruritus generally occurs more frequently in patients with advanced-stage disease, may accompany other systemic symptoms, may be more common in women, and is usually generalized.[52,53] Pruritus may be mild or severe enough that excoriations are produced from scratching. Proposed explanations of Hodgkin's-associated pruritus include cholestatic liver disease and peripheral sensory neuropathy. Pruritus typically resolves when the Hodgkin's disease is treated. [54]

Another unusual syndrome associated with Hodgkin's disease is alcohol-induced pain.[52] The pain usually begins within minutes of drinking alcohol and occurs in areas of nodal enlargement. Pain may also develop in the chest and radiate to the extremities or back. Alcohol-induced pain resolves with treatment of Hodgkin's disease; its mechanism is unknown.

### Laboratory Profile

Hematologic and chemical blood parameters show nonspecific changes that may correlate with disease extent. Abnormalities of peripheral blood counts may include neutrophilic leukocytosis, lymphopenia, eosinophilia, and monocytosis. At the onset of disease, the absolute lymphocyte count is usually normal in children, [55] although adults with extensive disease commonly have lymphopenia. Anemia may indicate the presence of advanced disease and usually results from impaired mobilization of iron stores.[56] Hemolytic anemia associated with Hodgkin's disease may be Coombs' positive and is accompanied by a reticulocytosis and normoblastic hyperplasia of the bone marrow.[57]

Several autoimmune disorders have been observed in association with Hodgkin's disease, including nephrotic syndrome, autoimmune hemolytic anemia, autoimmune neutropenia, and immune thrombocytopenia (ITP). ITP has been reported in 1% to 2% of cases of Hodgkin's disease and may occur in association with autoimmune hemolytic anemia.[58,59] and [60] Thrombocytopenia may develop before, at the same time, or after the diagnosis of Hodgkin's disease.[58,59] ITP frequently occurs in patients in remission after completion of therapy for Hodgkin's disease and is not usually associated with relapse. The treatment approach recommended for ITP in patients with Hodgkin's disease is similar to that in patients without malignancy. Response to ITP therapy is also similar.

The erythrocyte sedimentation rate, serum copper, and ferritin levels may be elevated, reflecting activation of the reticuloendothelial system. These nonspecific tests, if abnormal at diagnosis, may be useful in follow-up evaluation.[61]

### Immunologic Status

Patients with Hodgkin's disease exhibit immune system abnormalities at diagnosis that may persist during and after therapy.[62] In addition to the defects summarized in Table 23-4, natural killer cell cytotoxicity may be reduced in untreated patients.[55,62] Typically, enhanced sensitivity to suppressor T lymphocytes present at diagnosis

results in abnormal cellular immunity. After treatment, humoral immunity may be transiently depressed. *In vitro* studies have provided insights regarding the mechanism of immune dysregulation in Hodgkin's disease.[63,64] A variety of cytokine interactions have been proposed to explain the paradoxical presence of extensive inflammatory infiltrate, ineffective host antitumor response, and generalized cellular immune deficiency. [44]

| Activity | Untreated active disease | Disease-free survivors |
|---|---|---|
| Antigen-induced antibody production | Normal | Transiently depressed |
| Polymorphonuclear function | | |
| Chemotaxis | Decreased | Decreased |
| Metabolic reactivity | Decreased | Decreased |
| Delayed hypersensitivity skin tests | | |
| Recall antigens | Anergic | Reactive |
| Neoantigens | Anergic | Anergic |
| E rosette formation | Decreased | Decreased |
| Mitogen-induced T-cell proliferation | Decreased | Decreased |
| Mixed lymphocyte-induced proliferation | | |
| Autologous | Decreased | Decreased |
| Allogeneic | Slightly decreased | Slightly decreased |
| Sensitivity to suppressor monocytes | Enhanced | Enhanced |
| Sensitivity to suppressor T cells | Enhanced | Enhanced |
| CD4:CD8 ratio | Slightly decreased | Decreased |

From Slevin ML, Ellis TM, Newcombe JF, Fisher RI. The impact of Hodgkin's disease on the immune system. Semin Oncol 1980;7:67-73, with permission.

**TABLE 23-4. IMMUNE PROFILES IN HODGKIN'S DISEASE**

## DIFFERENTIAL DIAGNOSIS

Hodgkin's disease must be differentiated from other inflammatory causes of lymphadenopathy, particularly those with an indolent course (e.g., atypical mycobacterium infections and toxoplasmosis). NHL may have similar presenting signs and symptoms, but the growth rate of the affected lymph nodes is often more rapid than in Hodgkin's disease. In addition, NHL more frequently causes elevated uric acid or lactic dehydrogenase levels (see Chapter 24). Occasionally, biopsy of recurrent or persistent lymphadenopathy originally attributed to infectious mononucleosis or reactive hyperplasia yields the diagnosis of lymphoma. The cervical lymphadenopathy of Hodgkin's disease must also be differentiated from metastatic adenopathy of other primary tumors (e.g., nasopharyngeal carcinoma and soft tissue sarcoma).

A more difficult problem is that of a mediastinal mass that must be differentiated from normal thymus in an otherwise asymptomatic patient. The thymus is maximal in size in children aged approximately 10 years and may be differentiated from tumor on thoracic computed tomographic (CT) scans by its texture. [65] Ultimately, however, only biopsy can definitively confirm a diagnosis.

## DIAGNOSTIC WORKUP

Table 23-5 shows the recommended steps in the diagnostic workup of a child with Hodgkin's disease. An excisional lymph node biopsy is the preferred procedure to establish the diagnosis, as it permits evaluation of the malignant HRS cells within the background of characteristic architectural changes associated with the specific histologic subtypes. A careful physical examination with assessment of all node-bearing areas, including Waldeyer's ring, is essential, with measurement of enlarged nodes so changes can later be quantitated. If high cervical nodes are involved, a CT scan of the neck should be performed to evaluate Waldeyer's ring.

| |
|---|
| Physical examination with measurement of lymph nodes |
| Complete blood cell count with differential, erythrocyte sedimentation rate, renal and hepatic function tests, alkaline phosphatase level |
| Lymph node biopsy |
| Chest radiograph with measurement of mediastinal ratio |
| Computed tomography of neck and chest |
| Computed tomography or magnetic resonance imaging of abdomen and pelvis |
| Lymphangiogram[a] |
| Bone marrow biopsy[b] |
| Bone scan[c] |
| Gallium scan |
| Surgical staging with lymph node sampling[d] |

[a]Recommended for children with equivocal infradiaphragmatic imaging if expertise is available at center.
[b]Recommended for all children except those with stages IA/IIA.
[c]Recommended for children with bone pain and elevated alkaline phosphatase.
[d]Recommended in selected cases only.

**TABLE 23-5. DIAGNOSTIC EVALUATION FOR CHILDREN WITH HODGKIN'S DISEASE**

The chest radiograph provides preliminary information about mediastinal involvement and intrathoracic structures. Patients with “bulky” mediastinal lymphadenopathy measuring greater than or equal to 33% of the maximum intrathoracic cavity may benefit from a combined-modality treatment approach. The pulmonary parenchyma, chest wall, pleura, and pericardium are the most commonly involved extranodal sites of disease and should be further assessed by CT. Rostock and associates [66] found that approximately 50% of previously untreated patients had disease discovered on CT that had been missed on plain film, including pericardial or chest wall invasion, retrocardiac masses, and pulmonary parenchymal involvement. Although magnetic resonance imaging (MRI) is an effective tool for evaluating intrathoracic structures, the pulmonary parenchyma is best evaluated by thoracic CT.

The presence of infradiaphragmatic disease may be evaluated by CT, MRI, or lymphography (LAG). Administration of both oral and intravenous contrast agents is required to delineate accurately lymphadenopathy from other infradiaphragmatic structures by CT. Evaluation of the extent of abdominal and pelvic disease by CT scan is further complicated in children by the lack of retroperitoneal fat. Baker and colleagues [67] found that CT had a sensitivity of only 40% in detecting abdominal adenopathy. Standard MRI may provide better contrast resolution of infradiaphragmatic structures than does CT, and MRI provides better evaluation of fat-encased retroperitoneal lymph nodes.[68] LAG is unique in its ability to display the internal architecture of lymph nodes. This modality differentiates large normal reactive lymph nodes from those that contain tumor, and it is able to evaluate nodes too small for visualization by CT or MRI. However, LAG is more invasive and requires specific expertise to perform and interpret. If LAG is performed, the retroperitoneal lymph nodes remain opacified for months after the procedure, which permits monitoring of response to therapy by abdominal radiograph and facilitates design of infradiaphragmatic radiotherapy treatment portals.

Splenic involvement occurs in 30% to 40% of patients with Hodgkin's disease, and the size of the spleen may not correlate with the degree of disease involvement. Liver size and liver function studies are also unreliable indicators of hepatic disease. Organ size and degree of involvement do not strictly correlate because tumor deposits may be less than 1 cm in diameter and not visualized by diagnostic imaging modalities. Both CT and MRI scans may suggest splenic or hepatic involvement when these organs appear enlarged with areas of abnormal density. Because of the limitations of diagnostic imaging, only histologic assessment provides definitive evaluation of the spleen and liver. With advances in diagnostic imaging modalities and the standard use of systemic chemotherapy in contemporary pediatric treatment regimens, however, surgical staging of these organs currently has limited indications.

Nuclear imaging studies are often used in patients with Hodgkin's disease as a diagnostic and monitoring modality. Gallium-67 ( $^{67}$Ga) may be particularly useful in the evaluation of supradiaphragmatic Hodgkin's disease. Although this modality does not differentiate inflammatory lesions from lymphoma, the mediastinum demonstrates increased avidity in 60% to 70% of patients with previously untreated disease. In patients with mediastinal disease that has not completely regressed after therapy, persistent $^{67}$Ga avidity may indicate residual disease.[69] “Rebound” growth of the thymus after therapy may lead to increased thymic avidity for $^{67}$Ga, however.[70] $^{67}$Ga may also be useful in evaluating patients with normal physical examinations and recurrent systemic symptoms or abnormal laboratory values suspicious for disease recurrence. Positron emission tomography is currently under investigation as a diagnostic and monitoring tool for Hodgkin's disease.

Bone marrow involvement at the time of initial presentation of Hodgkin's disease is uncommon and rarely occurs as an isolated site of extranodal disease. The pattern of infiltration in the bone marrow may be diffuse or focal and is often accompanied by reversible marrow fibrosis. A bone marrow aspirate alone is not adequate to assess the marrow for disease. A bone marrow biopsy should be performed in any patient with clinical stage III to IV disease or B symptoms, or in any patient at the

time of disease recurrence. The exceedingly low yield of an abnormal bone marrow in a patient with newly diagnosed clinical stage I to IIA disease does not support its routine use during staging.

A technetium-99 bone scan with corresponding plain radiographs of abnormal areas aids in the assessment of skeletal metastases and should be reserved for the child who has bone pain, a serum alkaline phosphatase concentration elevated beyond that expected for age, or extranodal disease identified by other staging studies.

## STAGING

Hodgkin's disease appears to spread along contiguous lymph nodes until late in the course of disease. [52] The currently used Ann Arbor staging system, adopted in 1971, is based on this observation (Table 23-6).[71] The anatomic locations of lymph node chains designated as regions for the purpose of staging [72] are illustrated in Figure 23-4.

| Stage | Definition |
|---|---|
| I | Involvement of a single lymph node region (I) or of a single extralymphatic organ or site ($I_E$) |
| II | Involvement of two or more lymph node regions on the same side of the diaphragm (II) or localized involvement of an extralymphatic organ or site and one or more lymph node regions on the same side of the diaphragm ($II_E$) |
| III | Involvement of lymph node regions on both sides of the diaphragm (III), which may be accompanied by involvement of the spleen ($III_S$) or by localized involvement of an extralymphatic organ or site ($III_E$) or both ($III_{SE}$) |
| IV | Diffuse or disseminated involvement of one or more extralymphatic organs or tissues with or without associated lymph node involvement |

Note: The absence or presence of fever higher than 38°C for 3 consecutive days, drenching night sweats, or unexplained loss of 10% or more of body weight in the 6 months preceding admission are to be denoted in all cases by the suffix letters A or B, respectively.

**TABLE 23-6. ANN ARBOR STAGING CLASSIFICATION FOR HODGKIN'S DISEASE**

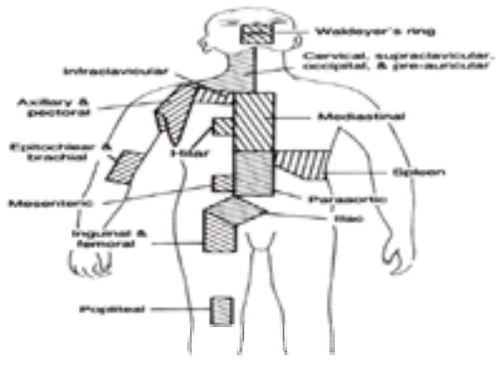



**FIGURE 23-4.** Anatomic definition of separate lymph node regions used for staging purposes. (Adapted from Kaplan HS, Rosenberg SA. The treatment of Hodgkin's disease. Med Clin North Am 1966;50:1591.)

The substage classifications A, B, and E amend each stage based on defined clinical features. Substage A indicates “asymptomatic” disease. B symptoms include fever exceeding 38°C for 3 consecutive days, drenching night sweats, and an unexplained loss of at least 10% of body weight over 6 months. Substage E denotes minimal extralymphatic disease; originally, this designation identified extralymphatic disease so limited that it could be subjected to definitive treatment by radiotherapy.[52] Disease limited to the spleen or splenic hilar or porta hepatis nodes has been said to have a more favorable prognosis relative to other stage IIIA disease and is designated IIIA1 (Fig. 23-5).[73]

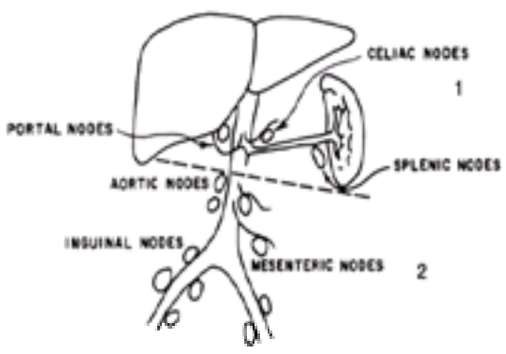



**FIGURE 23-5.** Diagram of lymph node regions in the abdomen showing the demarcation of stage $IIIA_1$ disease (nodal involvement limited to groups above the line) from stage $IIIA_2$ (nodal involvement below the line). (From Desser RK, Golomb HM, Ultmann JE, et al. Prognostic classification of Hodgkin's disease in pathologic stage III, based on anatomic considerations. Blood 1977;49:883, with permission.)

Pathologic staging, based on the findings of a staging laparotomy, including splenectomy, was routinely used in the 1970s to identify infradiaphragmatic disease. The importance of the staging laparotomy was emphasized after the discovery of an unusually high number of patients with clinically unsuspected splenic involvement at presentation.[74] In the 1980s, several factors brought about the widespread use of clinical staging: (a) advances in diagnostic imaging technology permitted more accurate evaluation of the retroperitoneal lymphatics, (b) the increasing use of systemic therapy for children precluded the need for confirmation of microscopic abdominal disease, and (c) the appreciation of infectious and neoplastic complications after splenectomy motivated the desire to maintain intact splenic function. Currently, surgical staging, most typically nodal sampling without splenectomy, is pursued only if the anticipated findings will significantly alter the treatment plan.

## TREATMENT

### Principles of Radiotherapy

The radiation responsiveness and radiocurability of Hodgkin's disease was recognized shortly after the discovery of x-rays. Even in the 1950s, when only orthovoltage equipment was available, investigators realized that patients with Hodgkin's disease could be treated effectively by irradiating all involved areas with high doses. With the development of megavoltage irradiation equipment—specifically the linear accelerator—Kaplan [52] devised techniques allowing treatment of large fields to high doses, with resultant cures. These pioneering efforts provide much of the basis for current standards of practice. In the early years, all patients, irrespective of age, were managed and treated alike. Not until 1970 was the first protocol devised specifically for the management of children. Today in the United States, the standard of care for the very large majority of children and adolescents with Hodgkin's disease is risk-adapted combined-modality therapy using low-dose, involved-field radiation in conjunction with multi-agent chemotherapy. An exception can be made for older adolescents and fully-grown patients with well-staged, favorable, nonbulky,

localized disease in whom high-dose, extended-field radiation alone can be curative. However, the desire to avoid radiation-related organ dysfunction and solid malignant neoplasms has resulted in the infrequent use of this treatment approach in the pediatric setting. Chemotherapy-alone protocols for children with early stage, nonbulky disease are under investigation.

The decisions regarding use of radiotherapy in the treatment program are a function of patient age, tumor burden, and concern about potential complications of treatment. All children with newly diagnosed Hodgkin's disease should be treated with curative intent. Good treatment planning with evaluation of sites of disease is an essential component of patient management.

***Radiotherapy Techniques***

The linear accelerator is the treatment machine of choice for the radiotherapy of Hodgkin's disease, with 6 mV the desired energy in most instances, particularly for supradiaphragmatic treatment. The 6-mV beam is sufficiently penetrating to produce reasonable beam homogeneity throughout an irregular treatment field, with most accelerator beams characterized by a reasonably flat beam profile. The point of maximal ionization of a 6-mV beam is close enough to the skin surface to avoid underdosing superficial lymph nodes, as in the neck. There remains a 10% to 20% dose inhomogeneity in a typical mantle field treated with a 6-mV beam, however, primarily from different separations in the superior and inferior portions of the field. Multiple off-axis site points should be measured and compensating filters used to minimize this dose in homogeneity. A beam energy greater than 6 mV may occasionally be useful for a large adolescent/young adult for subdiaphragmatic treatment.

A 4-mV linear accelerator is less desirable than a higher energy megavoltage machine. Many 4-mV accelerators have unusual beam profiles, especially with large irregular fields, in which the beam characteristics may be flat at a depth of 10 cm, but near the surface, the dose may be greater at the edge of the field than at the center. This characteristic, in combination with sites of lesser separation, such as the neck and axilla, may cause even greater dose heterogeneity requiring a compensating filter. Cobalt 60 units have the problem of dose falloff at the edge of a field, resulting in underdosing at the field edge. Additionally, the penumbra in a cobalt unit causes significant scatter radiation, which should be avoided in children. Orthovoltage is contraindicated. An extended source-to-skin distance is necessary to achieve large treatment volumes. Distances of less than 80 cm should be avoided because of poor depth-dose characteristics. Each field must be simulated and should be treated daily, five times per week. Appropriate gaps must be calibrated when adjacent areas are treated. Field edges should be permanently marked with tattoos.

Good immobilization is essential to the accuracy of radiotherapy. In very young patients who may move or in whom the fields may vary, a customized alpha cradle in conjunction with a chin band will improve reproducibility. For neck treatment, a face mask with a headrest will help assure a reproducible field. Children generally do not tolerate a bite block well. Arm position will vary according to the age of the patient. The akimbo arm position can be reproduced by simply having the child place his or her thumb in the waistband or belt.

Custom shielding blocks are essential. Divergent blocks are generally cast from a low-melting-point alloy such as Lipowitz metal (Cerrobend) and mounted on the collimator of the accelerator. These blocks are of a thickness calculated to reduce the transmitted beam by five half-values, which is approximately 3% of the prescribed dose. In addition to the 3% transmitted from the primary beam through the full-thickness Cerrobend block, there is some dose from radiation scattered internally from other tissues within the path of the beam. At the edge of the treatment field, the actual dose is approximately 50% of the prescribed dose with rapid dose falloff as the distance increases from the beam edge.

***Fields and Volumes***

An involved field includes not only the individual clinically involved or enlarged lymph nodes but also the surrounding lymph nodes within the same lymph node region, as delineated in Figure 23-4. A standard involved field is the minimum radiation field size used for Hodgkin's disease, and is appropriate in combined-modality programs. It is not acceptable to use a postage stamp–sized radiation field over an involved lymph node even in combined-modality therapy. The rare adolescent or young adult treated with radiation alone as the sole therapeutic modality is more appropriately treated with an extended field, subtotal lymphoid, or total lymphoid irradiation. For details regarding these fields, the reader is referred to a standard textbook of adult Hodgkin's disease. [75]

The mantle field is the most complex and important treatment field used in the management of Hodgkin's disease.[76] It encompasses the submandibular, submental, cervical, supraclavicular, infraclavicular, axillary, mediastinal, and pulmonary hilar lymph nodes. [52,77] The tumor volume may be enlarged to include the entire cardiac silhouette or lungs, or these organs may receive a lower dose through the use of a partial transmission block. [78] Because the mantle field treats a large volume, specific beam-shaping devices must be added. Blocks should be used over the occipital area, larynx, humoral heads, and spinal cord to avoid unnecessary morbidity. Axillary blocks may also be individualized. The “shrinking-field” technique is important when treating a patient with a large mediastinal mass, because as little as 10 to 15 Gy may lead to a dramatic response. Based on the response, blocks may be redesigned, thereby increasing protection to critical normal structures. An initial course of mantle irradiation in a symptomatic patient with extensive mediastinal disease often promptly relieves respiratory distress and enables the continuation of staging evaluation. Alternatively, one can use initial chemotherapy to provide tumor response and thus use a smaller radiation field for consolidative radiation in the situation in which one is faced with large mediastinal disease.

More common, when involved-field radiation is used, the mantle field is modified to exclude uninvolved areas. For example, the bilateral axillae are excluded when there is no initial axillary involvement ( Fig. 23-6). This is especially important in girls, for the axillary fields allow exposure to much of the breast, which should be avoided whenever possible. The modified mantle does not necessarily cover the entire neck and infraclavicular areas. When there is disease in the mediastinum, the modified mantle field includes the mediastinum, bilateral hila, and low neck, but not the mid- and high neck, unless these sites are specifically involved. The inferior border of the mediastinal involved field varies depending on the most inferior extent of disease but need not extend as low as the diaphragm, as is typical in the traditional mantle field. When the initial disease is located in the superior mediastinum, a modified mantle field would shield the pericardium and subcarinal areas for at least a portion of the treatment, allowing a 3- to 5-cm margin below the most inferior extent of disease. The minimantle field excludes the mediastinum entirely and is designed to treat the bilateral neck with or without the axillae. A Waldeyer's ring field ( Fig. 23-7) is used when the Waldeyer's ring structures or preauricular lymph nodes are involved, or when there is high cervical nodal disease that cannot be adequately covered by anterior/posterior opposed fields. It is a laterally placed field designed to cover the preauricular, postauricular, submental, retropharyngeal, occipital, parotid, submandibular and upper cervical areas. It is often matched to the upper border of an anterior/posterior opposed neck or mantle field at approximately the level of the thyroid notch. The Waldeyer's field may be treated with photons or electrons.

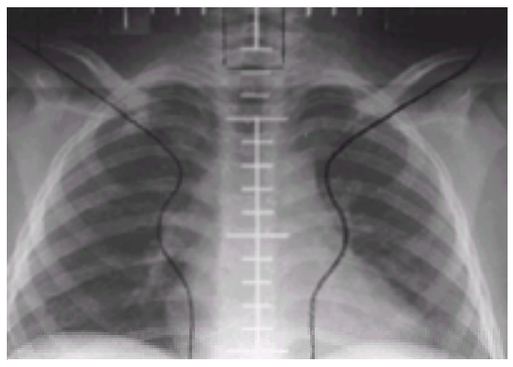


**FIGURE 23-6.** Modified mantle radiotherapy field, anterior port. Shaped blocks are used to contour the mediastinal-hilar silhouette and to treat the involved areas while shielding the uninvolved critical normal structures.

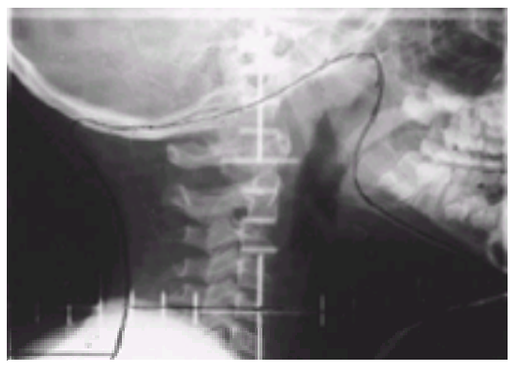


**FIGURE 23-7.** A Waldeyer's ring radiotherapy field involves a shaped field to treat the involved and adjacent lymph nodes in the upper neck areas, avoiding normal structures. Often, it is matched to a modified mantle field, as shown in Figure 23-6, using a split-beam technique.

It is unusual to have splenic disease without associated para-aortic disease. Thus, often these two areas are treated simultaneously when using involved-field radiation (Fig. 23-8). In this situation, a significant volume of the left kidney may be exposed with traditional opposing fields. The use of CT treatment planning is recommended, as it allows one to determine the volume of both the spleen and nearby left kidney. This allows one to contour the splenic field to avoid excess exposure to the left lung base, which otherwise might result in a left pleural base reaction or excess left kidney radiation. In cases of ipsilateral inguinal-femoral disease, a unilateral field covering these areas is appropriate. When there is ipsilateral iliac disease, the involved fields would be enlarged to cover all three sites as a hemipelvis field. When there is bilateral involvement of iliac, inguinal, or femoral areas, a pelvic field with gonadal shielding is used.

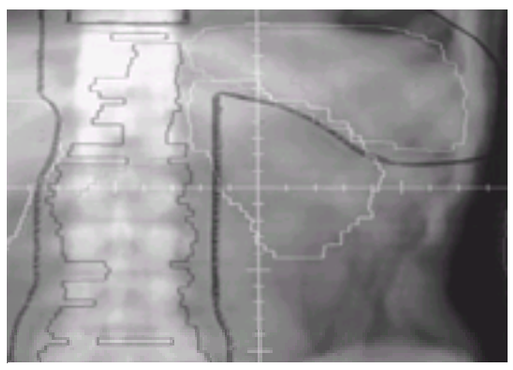


**FIGURE 23-8.** Spleen–para-aortic radiotherapy field as visualized on a digitally reconstructed radiograph taken from a three-dimensional conformal treatment plan. The spleen and kidneys are projected so the portal can be shaped and contoured.

In young women who require pelvic irradiation, transposition of the ovaries to a central midline position enables the use of a midline pelvic block to protect ovarian function.[79] The dose to the transpositioned ovaries can be decreased to 8% of that to the adjacent pelvic lymph nodes. [80] In male patients, a testicular shield minimizes the scatter radiation to the testes to 0.75% of the pelvic lymph node dose. [81] A conventional testicular shield is effective in postpubertal males; individual shielding devices must be fabricated for pubertal males.

In planned combined-modality programs, radiotherapy limited to involved sites requires judgment and flexibility. The involved field might better be termed a reasonable field. The goal with this approach is to treat the appropriate field to minimize retreatment match line problems in the event of a subsequent recurrence, and to minimize late effects. Table 23-7 provides functional definitions of involved radiation fields for various regions, but treating physicians must be flexible and use judgment considering the original site of disease and chemotherapy program used. The concept of involved-field radiation is most clear when dealing with stage I to II disease. For patients with stage III and select IV sites, the involved field could represent subtotal lymphoid irradiation or total lymphoid irradiation.

| | |
|---|---|
| Mantle | Supradiaphragmatic disease involving mediastinum as well as one or both cervical, supraclavicular, infraclavicular, or axillary lymph nodes. |
| Minimantle | Bilateral supramediastinal disease involving axilla, supraclavicular, infraclavicular, or cervical lymph node chains. |
| Hemiminimantle | Unilateral supramediastinal disease involving axilla, supraclavicular, infraclavicular, or cervical lymph node chains. |
| Mediastinum | Disease in mediastinum, one or both hila. |
| Para-aortic-splenic hilar-spleen | Subdiaphragmatic disease in spleen, splenic hilar, or para-aortic areas. |
| Spade | Subdiaphragmatic disease in spleen, splenic hilar, paraaortic, and iliac areas. |
| Pelvis | Disease in bilateral iliac and inguinal-femoral areas. |
| Hemipelvis | Disease in unilateral iliac and inguinal-femoral areas. |
| Inverted Y | Subdiaphragmatic disease in spleen, splenic hilar, para-aortic, and pelvic areas. |

**TABLE 23-7. INVOLVED-FIELD REGIONS**

### *Dose and Time Factors*

The radiation dose response curve has demonstrated that with a radiation dose of 36 to 40 Gy, local control exceeds 95%. For subclinical disease, doses of 30 Gy or greater provide local control in greater than 95% of patients. [82] However, for bulky disease, the control rate with radiation alone is less good and combined-modality therapy is needed. When combined-modality programs are used, lower radiation doses in the range of 20 Gy achieve a local control rate of 97%. [11] It is these data that prompted the use of low-dose, involved-field radiation with chemotherapy for children. The current pediatric protocols generally use between 15 and 25 Gy with chemotherapy, often with the radiation dose being determined by the response to chemotherapy. Residual radiographic abnormalities, particularly in the mediastinum, after a planned course of therapy are common and do not necessarily represent active Hodgkin's disease. A positive $^{67}$Ga scan at the end of therapy is rarely seen but raises concern of residual active disease, thus necessitating biopsy confirmation. A negative $^{67}$Ga has a lower predictive value, however, particularly in patients with stage III and IV disease. In one series of children and adults, the negative predictive value of $^{67}$Ga was only 64.5% for patients with stage III to IV disease, resulting in a relapse-free survival of only 48%. [83] Thus, a negative $^{67}$Ga scan does not necessarily mean the patient is disease free. The appropriate dose-time relationship in the treatment of Hodgkin's disease is less clear than the dose-response data. In light of normal tissue tolerance, patient acceptance, and tumor control, tumor doses of 1.5 to 1.8 Gy per day, five times a week, are most appropriate. When treating larger volumes, doses of 1.5 Gy are best tolerated, and no indication for using larger fraction sizes exists.

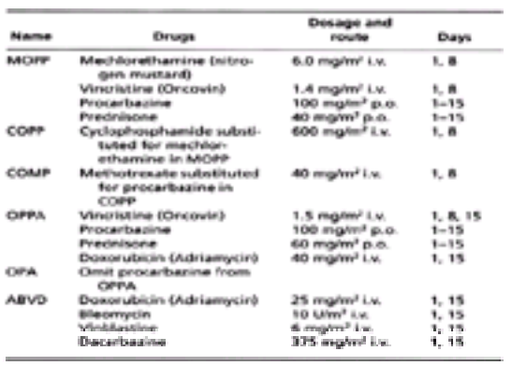
### Combination Chemotherapy

Current effective multidrug regimens for Hodgkin's and other pediatric malignancies combine non–cross-resistant agents with the following properties:

1. Each agent should be individually active against the tumor.

2. The agents should differ in the mechanism of antineoplastic activity, thereby targeting different cellular or biochemical events and preventing development of resistance.
3. Toxicities of the agents should not overlap, so that each drug can be administered at full single-agent dose.

The agents selected for the original combination therapy of Hodgkin's disease (MOPP) fulfilled these qualifications ( Table 23-8).[9] The MOPP combination, with agents delivered at full doses, produces long-term disease-free survival in approximately 50% of adult patients with advanced disease. [10] Additional maintenance therapy does not extend remission but rather predisposes to excess morbidity from higher cumulative exposures of agents with dose-related toxicity. Children with Hodgkin's disease have similar or better treatment outcomes after MOPP therapy compared to adult patients [11,12,13,14] and [15] but are also at risk for adverse MOPP-related sequelae including AML and infertility. Both toxicities are correlated with the cumulative doses of alkylating agent chemotherapy. The risk of secondary AML has been dramatically reduced by restricting cumulative doses of alkylating agent chemotherapy and substituting other, less leukemogenic alkylating agents (e.g., cyclophosphamide for mechlorethamine).[84] Similarly, the risk of infertility, which is almost universal in boys following treatment with six to eight cycles of MOPP therapy, may be reduced when treatment with gonadotoxic alkylating drugs, especially procarbazine, is limited to three cycles. [85,86]



| Name | Drugs | Dosage and route | Days |
|---|---|---|---|
| MOPP | Mechlorethamine (nitrogen mustard) | 6.0 mg/m² i.v. | 1, 8 |
| | Vincristine (Oncovin) | 1.4 mg/m² i.v. | 1, 8 |
| | Procarbazine | 100 mg/m² p.o. | 1–15 |
| | Prednisone | 40 mg/m² p.o. | 1–15 |
| COPP | Cyclophosphamide substituted for mechlorethamine in MOPP | 600 mg/m² i.v. | 1, 8 |
| COMP | Methotrexate substituted for procarbazine in COPP | 40 mg/m² i.v. | 1, 8 |
| OPPA | Vincristine (Oncovin) | 1.5 mg/m² i.v. | 1, 8, 15 |
| | Procarbazine | 100 mg/m² p.o. | 1–15 |
| | Prednisone | 60 mg/m² p.o. | 1–15 |
| | Doxorubicin (Adriamycin) | 40 mg/m² i.v. | 1, 15 |
| OPA | Omit procarbazine from OPPA | | |
| ABVD | Doxorubicin (Adriamycin) | 25 mg/m² i.v. | 1, 15 |
| | Bleomycin | 10 U/m² i.v. | 1, 15 |
| | Vinblastine | 6 mg/m² i.v. | 1, 15 |
| | Dacarbazine | 375 mg/m² i.v. | 1, 15 |

**TABLE 23-8. CHEMOTHERAPY REGIMENS FOR HODGKIN'S DISEASE (REPEAT CYCLE EVERY 28 DAYS)**

The development of the ABVD in the 1970s provided another effective non–cross-resistant chemotherapy regimen that did not produce an excess risk of secondary AML or infertility (Table 23-8).[87] Dose-related toxicity attributed to agents in the ABVD combination includes cardiomyopathy and pulmonary fibrosis, resulting from the doxorubicin and bleomycin, respectively. ABVD was initially used as salvage therapy for adult patients with MOPP-resistant disease, and later alternated with MOPP in an effort to enhance antineoplastic activity. [88] Subsequent studies in adult patients demonstrated that ABVD was superior to MOPP alone and had comparable efficacy to MOPP alternating with ABVD.[17] Superior treatment results and absence of leukemogenesis and permanent gonadal toxicity have made ABVD the preferred frontline regimen for adults with Hodgkin's disease. However, concerns regarding potential cardiopulmonary sequelae have restricted its use as the sole regimen in pediatric patients. Currently, ABVD, or similar hybrid combinations, are incorporated into risk-adapted treatment regimens prescribing fewer cycles of chemotherapy for children with localized, favorable Hodgkin's disease. In patients with advanced and unfavorable Hodgkin's disease, ABVD is more likely to be alternated with MOPP or similar hybrid combinations to improve disease control and reduce dose-related toxicity related to alkylating agent, anthracycline, and bleomycin chemotherapy.

### Treatment Results

Optimal therapy involves a multidisciplinary approach from the time of diagnosis. This is particularly important, as treatment decisions are currently based on risk features present at diagnosis, including the presence of B symptoms, stage, nodal bulk, and number of involved nodal regions. Assignment of stage and treatment are best determined after the pediatric and radiation oncologists have had the opportunity to examine the patient and review staging study results, preferably with simultaneous input from a diagnostic imager. In this way, a consistent plan for chemotherapy and radiation therapy can be presented to the family by all health care providers and reviewed periodically after response evaluations.

#### *Combined-Modality Therapy*

From the 1960s to 1980s, standard-dose (35 to 44 Gy), extended-volume radiation therapy was commonly used in conjunction with combination chemotherapy to enhance local tumor control. The observation of substantial late effects in children who received high-dose radiotherapy provided the impetus for studies evaluating combined-modality treatments prescribing low-dose (15.0 to 25.5 Gy), involved-field radiation therapy. [11,89] As more trials established that local disease control was maintained with reduced radiation, six cycles of non–cross-resistant combination chemotherapy evolved as the standard pediatric approach in combined-modality regimens. Table 23-9 and Table 23-10 summarize the results of major pediatric trials organized from the 1970s through the 1990s that established the efficacy of combined-modality therapy.[11,12,13] and [14,18,19,20,21,22,23] and [24,90,91,92,93,94] and [95]

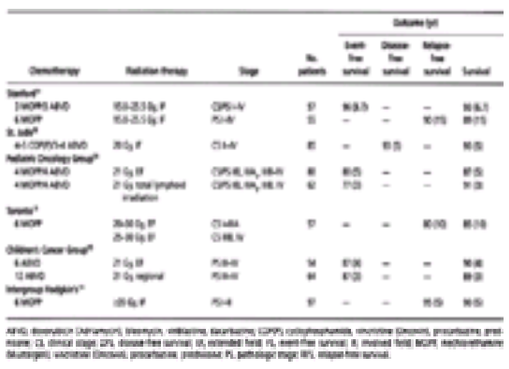


**TABLE 23-9. TREATMENT RESULTS OF NORTH AMERICAN PEDIATRIC COMBINED-MODALITY TRIALS**

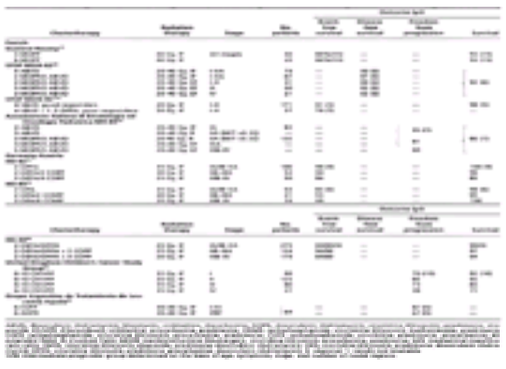


**TABLE 23-10. TREATMENT RESULTS OF EUROPEAN AND SOUTH AMERICAN PEDIATRIC COMBINED-MODALITY TRIALS**

### *Combined-Modality Therapy versus Chemotherapy Alone*

Multiple trials have established the efficacy of treatment with non–cross-resistant chemotherapy alone for pediatric Hodgkin's disease ( Table 23-11).[15,19,93,95,96,98,100,101,102,103,104 and 105,113] This treatment approach offers advantages for children managed in centers without access to radiation facilities, trained personnel, and diagnostic imaging modalities needed for clinical staging. The use of systemic therapy also avoids the potential long-term musculoskeletal complications, organ dysfunction, and solid tumor malignancies associated with high-dose, extended-volume radiation therapy.

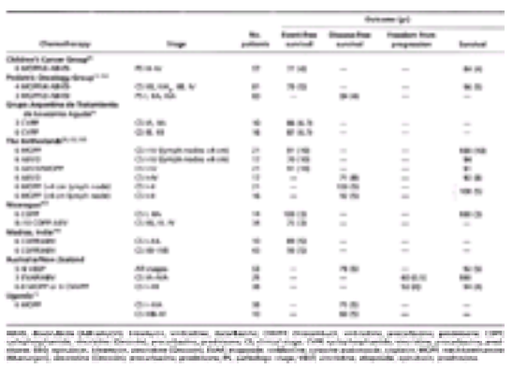


**TABLE 23-11. TREATMENT RESULTS OF PEDIATRIC CHEMOTHERAPY-ALONE TRIALS**

Earlier chemotherapy-alone trials prescribed 6 to 12 cycles of MOPP or hybrid therapies containing alkylating agents [ChlVPP (chlorambucil, vinblastine, procarbazine, and prednisone) and CVPP (cyclophosphamide, vinblastine, procarbazine, and prednisone)] in clinically staged children. [15,96,97 and 98] Acute hematologic and infectious toxicities associated with these treatments were acceptable and easily managed. Follow-up data regarding long-term toxicity have not been reported, although preliminary reports suggest a high frequency of gonadal toxicity in boys. [15,99]

Contemporary chemotherapy-only trials have used alternating non–cross-resistant regimens [MOPP/ABVD, COPP (cyclophosphamide, vincristine [Oncovin], procarbazine, prednisone)/ABV hybrid, CVPP/EBO (etoposide, bleomycin, and vincristine)] or combinations without alkylating agent chemotherapy [ABVD, EVAP (etoposide, vinblastine, cytarabine, and cisplatin)/ABV]. [100,101,102,103,104,105,106 and 107] Early results from these studies demonstrate treatment outcomes similar to those achieved with combined-modality therapy. Again, acute hematologic and infectious toxicities related to these regimens appear acceptable, but long-term cardiopulmonary sequelae have not been evaluated.

Evaluation of the efficacy of chemotherapy-only treatment regimens has been difficult because most reports describe outcome after nonrandom treatment assignments in small clinically staged cohorts. Some trials specifically exclude patients with unfavorable risk features based on bulk and number of involved nodal sites. Only a few randomized pediatric trials have compared treatment outcome after chemotherapy alone to combined-modality therapy. The Grupo Argentino de Tratamiento de Leucemia Aguda prospectively evaluated CVPP chemotherapy alone to CVPP plus involved-field radiotherapy. [107] Treatment outcomes after both regimens were comparable in stage I–II patients. Combined-modality therapy with CVPP plus radiation compared to CVPP chemotherapy alone, however, resulted in better disease-free survival in patients with localized unfavorable [more than two involved nodal areas, bulky peripheral (greater than 5 cm) and bulky mediastinal lymphadenopathy] and advanced stage disease. The next Grupo Argentino de Tratamiento de Leucemia Aguda trial demonstrated comparable event-free survival among patients with favorable disease presentations (as defined by a prognostic factor index based on age, presence of B symptoms, stage, and number of involved nodal regions) randomly assigned to three or six cycles of CVPP. [93]

North American pediatric cooperative group randomized trials have also prospectively compared treatment with combined-modality therapy to chemotherapy-alone. A Children's Cancer Group trial showed equivalent efficacy of 12 cycles of alternating MOPP/ABVD and six cycles of ABVD plus low-dose (21-Gy) involved-, extended-, or total-lymphoid radiation fields in children with pathologic stage III–IV Hodgkin's disease. [95] Although event-free and overall survival were not statistically different between the two groups, children randomized to combined-modality therapy showed higher 4-year event-free and overall survival rates (87% and 90%, respectively) compared to those who received chemotherapy alone (77% and 90%, respectively). A Pediatric Oncology Group study prospectively assessed the benefit of combining low-dose radiation therapy to eight cycles of alternating MOPP-ABVD in children with advanced-stage Hodgkin's disease. [94] Analysis of results based on treatment intent failed to show an advantage for combined-modality therapy. However, analysis based on treatment actually delivered showed a superior outcome in patients treated with chemotherapy and radiation therapy. Limited information is available regarding late treatment effects due to the brief follow-up period after the Children's Cancer Group and Pediatric Oncology Group trials, but one would anticipate higher risks of leukemogenesis, infertility, and cardiopulmonary toxicity related to the higher cumulative doses of alkylating agents, doxorubicin and bleomycin, prescribed in the chemotherapy-alone regimens compared to the combined-modality regimens.

In summary, several studies have demonstrated that chemotherapy alone is effective therapy for pediatric Hodgkin's disease. The advantage of this approach is the elimination of radiation-associated adverse sequelae including musculoskeletal maldevelopment, cardiac dysfunction, and solid tumor induction. Chemotherapy-alone treatment protocols rely on higher cumulative doses of agents with established dose-related toxicity, however, especially alkylating agent chemotherapy, which may contribute to acute and late treatment morbidity. The limited results of controlled randomized trials suggest that the addition of radiation therapy may improve outcome in children with unfavorable and advanced-stage Hodgkin's disease. However, further investigation is clearly needed to identify the prognostic features of patients who may benefit from the addition or omission of radiation therapy.

### *Risk-Adapted Therapy*

#### Favorable Clinical Presentations

In the 1990s, pediatric Hodgkin's trials confirmed that disease-free survival was not compromised by reducing the number of multi-agent chemotherapy cycles, radiation doses, and treatment volumes in clinically staged patients with “favorable” clinical presentations. [20,21,22,23 and 24,86,108,109] The favorable designation has varied among the individual studies but is typically characterized by localized nodal involvement in the absence of B symptoms and bulky disease. *Bulky* mediastinal lymphadenopathy is designated when the ratio of the maximum measurement of mediastinal lymphadenopathy to intrathoracic cavity on chest radiograph equals or exceeds 33%. Risk factors considered in other studies include the number of involved nodal regions, the presence of hilar adenopathy, the size of peripheral lymphadenopathy, and extranodal extension. Pediatric investigations of risk-adapted therapy for localized, favorable clinical presentations of Hodgkin's disease are summarized in Table 23-10.

#### Unfavorable Clinical Presentations

Clinical presentations are designated *unfavorable* when associated with the presence of B symptoms, bulky mediastinal or peripheral lymphadenopathy, extranodal extension of disease, and advanced (stage IIIB–IV) disease. Two standard treatment approaches have been used for pediatric patients with unfavorable clinical presentations. Conventional therapy prescribes non–cross-resistant chemotherapy derived from MOPP or ABVD administered on a twice-monthly schedule for a total of 6 months. COPP has more recently replaced MOPP because cyclophosphamide is less myelosuppressive and leukemogenic than mechlorethamine. [84] Low-dose (15.0 to 25.5 Gy), involved-field radiation therapy may be delivered between treatment cycles or, more commonly, following completion of chemotherapy to consolidate remission. Treatment results for unfavorable pediatric Hodgkin's disease using combined-modality therapy with conventional chemotherapy regimens [e.g., MOPP/ABVD, COPP/ABVD, OPPA (vincristine [Oncovin], procarbazine, prednisone, doxorubicin [Adriamycin])/COPP] are summarized in Table 23-9.

The second treatment approach uses the strategy of abbreviated dose-intensive multi-agent chemotherapy featured in adult trials, such as the MOPP/ABV hybrid combination and the Stanford V regimen.[110,111 and 112] Advantages of these regimens include reduced therapy duration and cumulative chemotherapy doses. Chemotherapy is administered at weekly intervals for a period of 3 to 5 months during which myelosuppressive agents are alternated with nonmyelosuppressive

agents. Consolidative radiation therapy, usually standard-dose in the adult setting, is administered to sites of bulky or residual disease. Ongoing trials of the North American pediatric cooperative groups support the feasibility of this approach combined with low-dose radiation therapy. Long-term follow-up is not yet available to evaluate efficacy and treatment sequelae.

Recent trials evaluating the substitution of non-alkylating agent chemotherapy (e.g., methotrexate or etoposide) as an alternative to alkylating agent chemotherapy demonstrated an inferior event-free survival among patients with unfavorable clinical presentations. [23,113,114] The results of several studies support the designation of an intermediate risk group, however, which includes patients with clinically localized disease (stages I–IIIA) with unfavorable presentation, including bulky lymphadenopathy or extranodal extension. Long-term results of the German Pediatric Oncology Group trials indicate that event-free survival has not been compromised with reduction from six to four chemotherapy cycles in children with these “intermediate” risk features. [23]

**Relapsed Disease**

Most relapses in patients with Hodgkin's disease occur within the first 3 years, although some patients may relapse as long as 10 years after initial diagnosis. The excellent outcome for the majority of children with Hodgkin's disease has limited opportunities for pediatric investigators to evaluate salvage therapy programs in large patient cohorts. Treatment and ultimate prognosis after relapse is largely dependent on the initial therapy type and time of relapse. As many as 50% to 80% of patients who relapse after radiation therapy alone can be salvaged with chemotherapy or combined-modality therapy. Standard multi-agent chemotherapy and radiation therapy may salvage 40% to 50% of children with 1-year or longer initial remissions, but subsequent treatment sequelae, including second malignancies, may reduce ultimate survival.[115] Patients who develop refractory disease during or within 1 year of completing therapy respond poorly to conventional salvage therapy, as do patients with multiple relapses. For these high-risk patients, consolidation with myeloablative therapy followed by hematopoietic stem cell transplantation (HSCT) provides the best opportunity for a durable remission. Several studies support the feasibility and efficacy of this approach, which produces overall survival rates ranging from 30% to 50% in children and adolescents with relapsed Hodgkin's lymphoma. [115,116] The acute morbidity and mortality associated with HSCT may be substantial and are influenced by previous therapy exposures in the often extensively pretreated patients. Transplant-associated mortality occurs in approximately 10% of patients and most commonly results from infectious, cardiopulmonary, or neoplastic complications. [115,117] Patients have been reported to be at risk for relapse as late as 5 years after HSCT, emphasizing the need for heightened surveillance to assure continued remission status and monitor for late treatment sequelae.

The use of HSCT as initial therapy remains controversial because of the overall excellent prognosis of children with advanced and unfavorable Hodgkin's disease. Consensus has not been established among investigators regarding prognostic features that justify the risks of this aggressive approach. Until these issues are further clarified, HSCT should be reserved for patients after relapse or for those who are refractory to primary conventional therapy, including alkylating agents. Similar to trials in adults with Hodgkin's lymphoma, cooperative group investigations are required to address the issues of prognostic factors, optimal conditioning regimens, and timing of stem cell transplantation in children.

Allogeneic bone marrow transplantation has also been used for relapsed Hodgkin's disease. [118] Allogeneic bone marrow transplantation is associated with a lower relapse rate, perhaps related to an immunologic effect against the tumor. The high treatment mortality after these transplants, however, results in event-free survival rates similar to patients treated with autologous transplants.

Novel therapeutic approaches for relapsed Hodgkin's disease are currently under investigation. Immunotoxin therapy targeting antigens expressed by HRS cells include CD25 (the IL-2 receptor) and CD30 (the Ki-1 antigen). Clinical trials to date have included only small numbers of patients. [45,46] The feasibility of adoptive immunotherapy with cytotoxic clones of T lymphocytes specific for EBV LMP1 continues to be explored for patients with EBV-associated Hodgkin's disease. [119]

**Acute Toxicity**

***Acute Radiation Effects***

The short-term side effects of irradiation are generally not serious (see Chapter 11). They are a function of the total dose delivered and the volume irradiated, with the most acute toxicity reported after high-dose, large-volume, radiation-only programs, which today are seldom used in children. Low-dose, involved-field radiation as used in combined-modality treatment programs is well tolerated. Potential toxicity from the radiation component of the combined-modality program may include moderate erythema, hyperpigmentation, or both, of the irradiation skin. There may be transient partial hair thinning at the occiput from a high neck radiation field. Mild gastrointestinal disturbance may occur and possible alteration in taste or xerostomia if a large Waldeyer's ring field is required to a moderately high dose. Granulocytopenia and thrombocytopenia may occur but usually reflect bone marrow suppression from prior chemotherapy. Lhermitte's syndrome, a sensation of an electric shock radiating down the back and into the extremities on flexion of the neck, is rare, self-limited, and does not represent a prodrome of later neurologic dysfunction. In general, acute radiation effects are self-limited and reversible.

***Immediate Effects of Chemotherapy***

Multi-agent chemotherapy programs can cause nausea and vomiting. The efficacy of serotonin receptor antagonist antiemetics, such as ondansetron, greatly improve tolerance to chemotherapy. Anticipatory nausea, which commonly occurs in teenagers, often responds to premedication with benzodiazepines. Nitrogen mustard, vincristine, and doxorubicin may cause severe local tissue damage if infiltrated into the subcutaneous tissues. Vinblastine and dacarbazine may cause a local burning sensation as they are injected. Many chemotherapy programs produce some degree of reversible alopecia. The neurotoxicity of vincristine, the cardiac toxicity of doxorubicin, and the pulmonary toxicity of bleomycin, as well as other side effects, are discussed in Chapter 10. A thorough review of the toxicities of individual chemotherapy agents should be made before any are administered either alone or in combination.

***Infection***

The most common dose-limiting acute toxicity of multi-agent chemotherapy is myelosuppression. Courses of treatment may need to be delayed because of low blood counts. It is preferable to give therapy on schedule at full doses and thus the use of transfusion or simulating factors may be needed for patients receiving aggressive therapy for advanced disease. Some patients may require hospitalization for antibiotic therapy if they develop fever during a period of neutropenia.

The risk of serious bacterial infection, once attributable to splenectomy associated with staging laparotomy, [120] is less frequently observed now that surgical staging is seldom performed. Patients who have undergone a prior splenectomy or splenic radiation, however, should be given a prophylactic antibiotic regimen and guidelines to follow during febrile illnesses. In addition, vaccines available against pneumococcus, *Haemophilus influenzae,* and meningococci may further decrease the risk of serious bacterial infection for these patients, although sustained antibody titers have not been achieved in patients with Hodgkin's disease. [121]

Herpes zoster and varicella infections are seen in 35% of children with Hodgkin's disease. The frequency is directly related to the intensity of treatment. [122] Prompt administration of antiviral therapy has reduced the severity and morbidity of these infections. The management of immunosuppression with these and other infections is discussed in Chapter 40.

**Late Effects**

***Soft Tissue and Bone Growth Alterations***

Early reports described a disproportionate alteration in sitting height compared with standing height among a group of children who received axial skeletal radiation. [123] A follow-up study demonstrated that prepubertal children who received high-dose radiotherapy (greater than 33 Gy) to the entire spine experienced a significant height loss.[124] Pubertal and postpubertal children given similar treatment and prepubertal children given less than 33 Gy to more restricted radiation volumes did not show clinically significant height impairment. [124] Other investigators have confirmed that lower doses of involved-field radiation used with chemotherapy are most probably not associated with clinically significant growth retardation. [48]

Other musculoskeletal abnormalities observed after high-dose radiation include narrowing of the thoracic apex, intraclavicular narrowing with symmetric shortening of the clavicles, and atrophy of the soft tissues of the neck. Rare sequelae include retroperitoneal fibrosis and brachial plexopathy. Avascular necrosis of the femoral

head has been seen with corticosteroid use, but high-dose radiation may also be a contributing factor. The current treatment protocols with lower dose and smaller volumes of radiation are expected to lessen or eliminate the marked changes seen in young patients treated in the past.

### *Pulmonary Sequelae*

Acute and chronic pulmonary complications reported after therapy for Hodgkin's disease include radiation pneumonitis, pulmonary fibrosis, and spontaneous pneumothorax, although these sequelae are uncommon and are a result of therapy given years ago. [89] However, more current pediatric Hodgkin's disease therapy using radiation therapy and ABVD have shown a significant incidence of asymptomatic pulmonary dysfunction after treatment, which appears to improve with time.[90,91,125,126] However, grade 3 and 4 pulmonary toxicity has been reported in 9% of children receiving 12 cycles of ABVD followed by 21-Gy radiation. [95] In addition, ABVD-related pulmonary toxicity may result from fibrosis induced by bleomycin or “radiation recall” pneumonitis related to administration of doxorubicin. Pulmonary veno-occlusive disease has been observed rarely and has been attributed to bleomycin chemotherapy. [127] This incidence of veno-occlusive disease may be underreported, as some cases have been misdiagnosed as pulmonary fibrosis.

### *Cardiovascular Sequelae*

Both radiation therapy and chemotherapy used in the treatment of Hodgkin's disease may have toxic effects on the heart and blood vessels, although symptomatic sequelae are uncommon. They include cardiomyopathy with congestive heart failure, acute pericarditis, pericardial effusion, chronic constrictive pericarditis, coronary artery disease with myocardial infarction, conducting system abnormalities, valvular dysfunction, and peripheral vascular disease. Reduction of anthracycline chemotherapy doses and radiation doses and volumes in contemporary pediatric regimens have diminished the frequency of acute toxicity. However, delayed subclinical cardiovascular injury and its effect on the progression of degenerative cardiovascular disease is just now becoming apparent as survivors of pediatric Hodgkin's disease enter their third and fourth decades.

Cardiac injury from radiation is also related to dose, volume, and fraction size. Both the pericardium and myocardium may be affected. The spectrum of cardiac dysfunction ranges from asymptomatic radiographic abnormalities to life-threatening illness (e.g., constrictive pericarditis requiring pericardiectomy). The incidence of cardiac injury after high-dose mantle irradiation is approximately 13% in both children and adults. Arterial vascular injury, including underdevelopment of the great vessels, coronary artery disease with coronary fibrosis, and accelerated atherogenesis, has been observed in long-term survivors of Hodgkin's disease treated with high-dose mantle irradiation.[89] Clinically apparent cardiac disease occurs infrequently in survivors of Hodgkin's disease treated during childhood or adolescence with modern radiotherapy techniques. However, morphologic abnormalities of screening echocardiograms observed in high frequency in some pediatric cohorts emphasize the importance of longitudinal follow-up to determine the incidence of clinically significant pericardial disease and myocardial dysfunction in this age group. [128,129] and [130]

In a series of survivors of childhood Hodgkin's disease from Stanford, the actuarial risk of cardiac disease requiring pericardiectomy was 4% at 17 years. [131] Patients with severe pericardial complications received little or no cardiac blocking, and most were irradiated before the introduction of subcarinal blocking. The recognition of radiation-induced pericardial disease described in this and other series has resulted in the modification of irradiation techniques to reduce the dose of radiation to the heart.[132] The rates of acute pericarditis and effusion have declined in later cohorts in which patients received partial cardiac shielding and subcarinal blocking, [133] confirming that risks for radiation-related significant pericardial disease are attributed to the total cardiac dose and volume.

Premature coronary artery disease and acute myocardial infarction have also been reported in pediatric Hodgkin's disease survivors. [131,134] Young age at diagnosis increases the risk. In the Stanford series, there is a 45-fold excess mortality risk from acute myocardial infarction in patients who received mediastinal irradiation in doses exceeding 30 Gy before age 20 years.[131] With lower volumes of radiation and protective cardiac shielding, the risk of radiation-related cardiac injury is greatly diminished. The Stanford cohort of 192 children treated with combined-modality treatment using lower doses and volumes of radiation reveal no death from myocardial infarction. Other cardiovascular sequelae, including arterial vascular injury, underdevelopment of the great vessels, coronary artery disease with coronary fibrosis, and accelerated atherogenesis, have been observed in long-term survivors of Hodgkin's disease treated with high-dose mantle irradiation. [89] The true incidence after modern treatment may be difficult to determine, as appropriate radiation treatment plans now prescribe low doses and volumes of mediastinal radiation and protective cardiac shielding.

The most common chemotherapeutic agents implicated in the development of cardiovascular complications in patients with Hodgkin's disease include the vinca alkaloids, alkylating agents, and anthracyclines. Raynaud's disease is an uncommon toxicity observed in some patients. This phenomenon appears to be caused by irreversible microvascular injury in patients treated with combination of vinblastine and bleomycin. Rarely, myocardial infarction in Hodgkin's patients has been attributed to vinca alkaloid therapy. Proposed mechanisms for this complication include ischemia resulting from coronary artery spasm or hypercoagulability. Although high cumulative doses of cyclophosphamide have resulted in adverse effects on the myocardium, these have largely been in the setting of HSCT in adults. Conventional doses of cyclophosphamide may exacerbate either anthracycline- or radiation-induced cardiac injury.

The cardiac dysfunction most commonly occurring after chemotherapy is related to anthracycline therapy, particularly with doxorubicin. Acute toxicities observed are relatively common and include sinus and supraventricular tachycardias and premature ventricular complexes. More serious arrhythmias, such as complete heart block, ventricular tachycardias, and sudden death, are uncommon. These abnormalities bear no relationship to chronic cardiomyopathy. Congestive heart failure with pericardial effusion and diffuse myocardial injury may occur acutely or as a chronic event with progressive failure associated with early mortality. The incidence of congestive heart failure increases with the cumulative doxorubicin doses greater than 550 mg per $m^2$ in adults. Young children may be more sensitive to anthracycline injury because of its adverse effect on cardiac myocyte growth.[135] In addition, children have a high frequency of anomalies of afterload and contractility. [135] Mediastinal radiation and other chemotherapies are thought to lower the threshold cumulative doses to the range of 350 to 400 mg per $m^2$. The risk of cardiotoxicity may also be impacted by the schedule of administration of doxorubicin. Schedules using smaller weekly doses or continuous infusion are associated with a lower incidence of congestive heart failure than are large doses given every 3 weeks. Factors associated with late cardiac decompensation include childbirth, viral infections, isometric exercises, alcohol and drug ingestion, and growth hormone–induced growth spurts. With current protocols using three to four cycles of ABVD and total cumulative doxorubicin doses of 150 to 200 mg per $m^2$and low-dose radiation, the incidence of clinically symptomatic cardiac disease is low. [90,91] Nevertheless, all patients treated with doxorubicin-containing chemotherapy and cardiac radiotherapy should be followed systematically for potential cardiac injury.

### *Endocrine Sequelae*

Using an elevated thyroid-stimulating hormone (TSH) level to define hypothyroidism, the incidence of thyroid dysfunction in irradiated patients has ranged from 4% to 79%. The sensitivity of the preadolescent thyroid may be higher than that of the adult gland. The iodine load of a pre-radiotherapy lymphangiogram has also been implicated as a factor in thyroid damage. The dose of radiation is important; only 17% of children who received neck irradiation of less than 26 Gy developed thyroid abnormalities compared with 78% in children who receive 26 Gy or more. In this series, investigators noted improvement in 36% of biochemically compensated hypothyroid children with time.[136] Thyroid nodules, hyperthyroidism, and thyroid cancer have been observed in patients treated for Hodgkin's disease. TSH and free thyroxine levels should be checked annually in patients who have received radiotherapy to the neck, and children who have elevated TSH levels should receive thyroid replacement therapy to reduce stimulation from prolonged TSH elevation.

Sterility, alterations in fertility, and potential gonadal injury after staging and treatment are important issues that should be addressed at the time of diagnosis and before therapy is instituted.[137] Pelvic radiation carries a high likelihood of ablation of ovarian function. The technique of oophoropexy, with transfer of the ovaries to a midline location, has allowed the preservation of ovarian function in young women with Hodgkin's disease. [80]

The younger the woman at the time of therapy, the higher the probability of maintenance of regular menses after therapy. When chemotherapy and radiation are used, the potential exists for increased risk of ovarian failure, although in the Stanford series, 87% of girls with Hodgkin's disease had normal menstrual function at long-term follow-up.[138] Normal pregnancies can occur after oophoropexy and pelvic radiation with no increased risk of fetal wastage or spontaneous abortion. [138,139] Of the pregnancies carried to term, no increase in birth defects was observed when compared with the offspring of sibling controls.

Treatment for Hodgkin's disease may carry a considerable risk of premature menopause related to the specific treatment modality and age at the time of therapy. Byrne and colleagues[140] reported a significantly increased relative risk of menopause during the early 20s in young women treated with either radiation alone or alkylating agents alone. The group at highest risk comprised women treated with alkylating agent chemotherapy and subdiaphragmatic radiotherapy (27-fold); 42% of these women had reached menopause by age 31 years compared to 5% for controls. The Stanford group has not observed an excessive risk of premature

menopause among young women with early-stage disease treated with radiation alone in doses of 40 to 44 Gy to radiation fields that omit the pelvis, however. [141]

The sterility issue in males is of much greater severity. Primary gonadal dysfunction may exist at the time of diagnosis of Hodgkin's disease in 30% to 40% of patients,[142] but despite this, pretreatment storage of sperm should be considered in older patients. High-dose radiation to the pelvis or a standard inverted-Y field may be associated with a transient oligospermia or azoospermia; however, recovery of function is common. [88] Six to eight cycles of MOPP and ChlVPP chemotherapy are associated with azoospermia in 80% to 90% of adult or pediatric males. [143,144] and [145] A high and dose-related incidence of testicular dysfunction has also been reported in pediatric patients treated with OPPA or OPPA/COPP, which is the result of the gonadotoxic agent procarbazine. [146] Anthracycline-based regimens, such as ABVD, are associated with a lower incidence of testicular damage: approximately 30% of males. [16,140,143] This azoospermia is transient with full recovery of spermatogenesis. Recovery of spermatogenesis has also been reported 10 to 15 years after documented azoospermia in boys after six cycles of MOPP in childhood [138] and in 50% of adult males after two to three cycles of MOPP.[85] The etoposide-based regimen of OEPA [vincristine (Oncovin), etoposide, prednisone, and doxorubicin] also has been used in boys with pediatric Hodgkin's disease.[147] Testicular function was normal in 27 boys receiving two cycles. However, when two to four cycles of COPP were added, the basal follicle-stimulating hormone levels rose to abnormal levels. The hormone-producing cells of the testes are more resistant to the effects of treatment than are the spermatogonia, so boys continue to grow and develop normally. Current treatment approaches are risk adapted in an effect to spare those patients with favorable and early-stage disease from the gonadotoxic sequelae, which result from the chemotherapy treatment programs needed to cure more advanced and unfavorable stage disease.

***Second Malignant Tumors***

The development of a second malignant neoplasm is the most serious late treatment complication among Hodgkin's disease survivors. The most common risk factors, particularly in those treated during childhood, are potential genetic influences and the type of treatment received. Newly acquired knowledge of genetic predisposition and secondary carcinogenesis related to tumor suppressor genes such as *RB1* and *p53* has greatly augmented our understanding of cancers. Among survivors of Hodgkin's disease, the association between malignancy and treatment for Hodgkin's disease has been given more attention than the genetic influences, predisposing survivors to a second malignancy. Possible genetic influences are of particular importance in light of the current knowledge of *BRCA1* and *BRCA2* genes with specific solid tumors. In addition, Hodgkin's disease is accompanied by defective cellular immunity, which may predispose to the development of a new malignancy. As well, it is important to consider causes of mortality among the population of interest. In the Stanford experience of 694 children and teenagers followed for up to 31 years after treatment of Hodgkin's disease, recurrent Hodgkin's disease accounted for greater than one-half of all deaths, accidents and other causes for one-fourth, and secondary cancers for one-fifth.[149]

Secondary acute nonlymphocytic leukemia (s-ANLL) and its precursor—a pancytopenic myelodysplastic syndrome—represents the most common second cancer seen in these patients. A standardized incidence ratio (SIR) (calculated as the ratio of observed to expected cases, or relative risk) for any leukemia of 78.8 [95% confidence interval (CI), 56.6 to 123.2) and an SIR of 321.3 (95% CI, 207.5 to 467.1) for acute myeloid leukemia/myelodysplastic syndrome have been reported from the Late Effects Study Group (LESG) for children younger than 16 years treated with chemotherapy.[150] This SIR for ANLL was reduced to 122 (95% CI, 36 to 254) with lower cumulative doses of alkylating agents and the omission of mechlorethamine as reported by German/Austrian investigators. [84]

The use of ABVD in lieu of MOPP and its derivatives greatly reduces the leukemia risk. [151] However, the epipodophyllotoxins and nitrosoureas are associated with s-ANLL.[152] The leukemia risk after radiation alone is extremely low (zero probability in LESG experience).

The peak frequency for secondary leukemia is within the first 5 to 10 years after treatment. [153] Some investigators have described a higher frequency of s-ANLL in splenectomized adults with Hodgkin's disease and have suggested that the spleen has a protective role, [152,154] whereas other investigators have found no such effect in adults,[155,156] or in children younger than 16 years when treated. [157]

The risk for NHL is also increased with an SIR of 20.9 (95% CI, 7.7 to 42.0) in the LESG experience. [150] An SIR of 15 (95% CI, 4.9 to 35.0) is reported for children younger than 20 years from five Nordic countries. [158] The risk of NHL may be related to overall immunosuppression associated with the disease as well as its treatment.

A variety of secondary solid tumors have been observed in patients treated for Hodgkin's disease. The risk of a secondary solid tumor escalates with the passage of time after diagnosis of Hodgkin's disease, with a latency of 20 years or more. The SIR of a solid tumor after treatment for Hodgkin's disease in the LESG data is 11.8 (95% CI, 8.7 to 15.4).[150] The most common solid tumors in that report were breast (SIR 75.3; CI, 45 to 118), thyroid (SIR, 32.7; CI, 15 to 55), bone (SIR, 24.6; CI, 6.4 to 54.5), brain (SIR 10.5; CI, 3 to 23), colorectal (SIR, 38.9; CI, 7.3 to 95.0), and stomach (SIR, 121.3; CI, 11.4 to 145.0). Similarly the Stanford experience in individuals younger than 21 years of age revealed increased risks for sarcoma, melanoma, breast, lung, thyroid, salivary gland, and stomach. [149] Nonmelanotic skin cancers, including basal cell cancers, are also common. Other institutions have reported similar data. [148,152,159,160] and [161] Several investigators have shown that age at treatment has a major effect on risk of second malignant tumors after treatment for Hodgkin's disease, and the increased risk of solid tumors in adolescent and young adults decreases as these individuals grow older. [152] The risks of secondary solid tumors vary by treatment and are largely related to radiation and radiation plus chemotherapy.[152] The risks greatly increase after relapse of Hodgkin's disease, thus requiring aggressive retreatment. [152,162]

Much attention has been given to the significant risk of secondary breast cancers in young women. [149,160,163,164] and [165] This risk increases with follow-up. The risk is highest for girls who received radiation at age younger than 15 years and declines with increasing age. There is no increased risk in women older than 30 years at the time of radiotherapy.[166] A high dose of radiation is a risk factor, with most breast cancers arising after 40 to 46 Gy of mantle irradiation, which includes treatment to the axilla.[166]

The quantitative estimate of risk of secondary cancers after pediatric Hodgkin's disease varies, depending on the length and completeness of follow-up. The updated Stanford experience of secondary cancers is especially valuable, as it contains a large number of patients with consistent and thorough long-term follow-up, with a median of 14.4 years (range, 1 to 37) as shown in Table 23-12.[167] This experience reviews 694 pediatric patients with 82 malignancies occurring in 78 patients. The data are compared with the 1994 Surveillance, Epidemiology, and End Results program cancer statistics. The estimated number of excess breast cancers per 10,000 person-year (absolute risk) is 63.1. These data highlight the importance of long-term monitoring of these patients, as the highest reported risks appear in series with the longest follow-up.

| Variable | Observed/ expected | Relative risk | Person-years |
|---|---|---|---|
| All cancers, female | 51/3.16 | 16.1 | 4,211 |
| All cancers, male | 27/3.18 | 8.49 | 5,197 |
| Leukemia | 8/0.25 | 32.3 | 9,657 |
| Non-Hodgkin's lymphoma | 3/0.49 | 6.09 | 9,657 |
| Breast cancer, female | 28/0.85 | 32.8 | 4,301 |
| Sarcoma | 15/0.11 | 135.0 | 9,637 |
| Other solid tumors | 27/6.52 | 4.1 | 9,530 |

**TABLE 23-12. RELATIVE RISK OF SECOND CANCER AFTER PEDIATRIC HODGKIN'S TREATMENT AT STANFORD**

Importantly, the risk of second malignant tumors comes from treatment given many years ago and does not reflect contemporary therapy. Thus, with the current recommended treatment of risk-adapted multi-agent chemotherapy and low-dose, involved-field radiotherapy, the risk of second cancers is expected to be substantially lower than that reported in the past.

Many groups have shown that the highest risk of complications of treatment is relapse of the Hodgkin's disease itself. [149,168] Thus, the goal for pediatric Hodgkin's

disease must continue to be curative treatment with initial therapy, resulting in the overall treatment that carries with it minimal morbidity and the highest quality of life.

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# MALIGNANT NON-HODGKIN'S LYMPHOMAS IN CHILDREN

IAN T. MAGRATH



## NATURE OF MALIGNANT LYMPHOMAS

Most cancers are neoplastic proliferations of an organ or tissue and therefore originate in a circumscribed anatomic location and spread from the point of origin by local invasion or by the process of metastasis. Malignant lymphomas differ radically from this pattern. They are neoplasms of the constituent cells of the immune system, cells that normally circulate throughout the body to subserve their functions. Therefore, with some exceptions, lymphomas are generalized diseases from the outset and have patterns of spread that mimic the migration patterns of normal lymphoid cells.[1] Malignant transformation can occur in any of the functionally different subpopulations of lymphoid cells, or, more likely, the precursors of these subtypes. Consequently, all except neoplasms of natural killer (NK) cell origin will manifest clonal rearrangements of immunoglobulin or T-cell receptor genes (clonal, because of the need for multiple molecular abnormalities to occur in the same cell, or its progeny, for full neoplastic transformation to occur) and will also express many of the immunophenotypic characteristics of a specific lymphoid subset, for a block to differentiation appears to be a general feature of lymphoid neoplasms. Sometimes, aberrant differentiation may occur (e.g., the expression of myeloid antigens or rearrangement of both immunoglobulin genes and T-cell receptor genes in immature lymphoid neoplasms). There are several possible explanations for this phenomenon. There may have been a failure to inhibit differentiation along a specific pathway, or, in the case of biphenotypic characteristics, aberrant expression of a small number of genes because of molecular genetic aberrations. Alternatively, transformation may have occurred in cells that are continuously generated in normal individuals only to be eliminated by apoptosis (but inhibited from undergoing apoptosis by pathologic molecular events).

It should not be assumed that all of the molecular abnormalities present in the cell occurred at the same point in its differentiation. Indeed, cells at different levels of differentiation may be more or less susceptible to specific molecular abnormalities. For example, translocations involving immunoglobulin or T-cell receptor genes are likely to occur during or close to the point in normal lymphoid cell differentiation when these genes undergo rearrangement to generate functional T- or B-(immunoglobulin) cell receptors. The molecular genetic events that lead to lymphoid neoplasia are, thus, best understood in the context of the normal events occurring during lymphocyte development (ontogeny), whereas the clinical features of each lymphoid neoplasm will, to a greater or lesser degree, reflect the behavior of its normal counterpart cell or cells.[1,2] Our understanding of lymphoid neoplasia will continue to increase *pari passu* with our knowledge of normal lymphocyte ontogeny, lymphocyte function, and the derangements of molecular pathways brought about by pathologic genetic changes.

Histopathologic evaluation, including immunophenotyping, remains the primary and most widely used means of classifying the lymphoid neoplasms, but the most precise diagnosis is likely to be made when the identification of the associated nonrandom molecular abnormalities is added to these standard diagnostic modalities. It is these molecular abnormalities, in the setting of the intracellular milieu on which they are superimposed, that ultimately determine the characteristics of the tumor cells. It is because the molecular genetic abnormalities are “context specific” that they are not exclusively associated with a specific neoplasm, although this frequently is the case. Thus, the mere presence of a single genetic lesion is not always sufficient to establish a diagnosis. For example, follicular and large B-cell lymphomas (LBCLs) may both contain 14;18 translocations. The former, however, a rare tumor in children, can also evolve into the latter, suggesting at least a close relationship between neoplasms with the same molecular lesions, even if they are histologically and clinically quite different. Conversely, the same functional end result can be achieved by quite different genetic lesions. For example, the same gene may be deregulated by a process of chromosomal translocation—in some cases by several different chromosomal translocations—by other forms of chromosomal rearrangement, such as amplification or deletion, or by point mutation. Alternatively, the altered expression of genes that lie on the same molecular pathway or interact with the same molecular pathway, or even the expression of viral genes in a cell, can create similar or identical functional end results.

Just as the consequences of genetic lesions are dependent on the cell in which they occur, so, too, the genetic lesions themselves (particularly chromosomal aberrations) tend to be cell-type, or at least lineage specific. This presumably reflects the likelihood of a given genetic abnormality arising (which is influenced by both chromatin and gene expression patterns) or its ability to create neoplastically relevant functional changes within a given cell type. Some genetic lesions, however, can arise in a broad variety of cells (e.g., p53 mutations). Such lesions may contribute to a variety of cancers because of a pivotal role in the life cycle of the cell—they are frequently involved, as in the case of p53, in cell proliferation or apoptosis (programmed cell death). Apoptosis is a critical element of both lymphocyte ontogeny (only functionally competent cells are permitted to survive) and of the immune response (with respect to both the regulation of the expansion of lymphoid and other cell populations and the killing of cells targeted by the immune response). Indeed, malfunction of genes involved in apoptotic pathways is robably an essential element of all carcinogenic events and is likely to be essential to the survival of cells containing genetic lesions that would otherwise cause them to be diverted to an apoptotic pathway. The inhibition of apoptosis may itself contribute to the stepwise accumulation of genetic lesions in the process of lymphomagenesis. The need for inhibition of apoptotic pathways in the course of carcinogenesis is emphasized further by the fact that genes involved in cell proliferation, such as c- *myc*,[3] are invariably coupled to apoptotic pathways, doubtless as part of the regulatory mechanisms that govern normal cellular proliferation.

Lymphomas, then, are neoplasms of lymphocytes or their precursors, which arise as a consequence of genetic aberrations that influence their proliferation, differentiation, and ability to undergo apoptosis. To a varying extent, however, neoplastic cells remain sensitive to the signals received by normal cells, which govern these processes, as well as their migration. Tumor cell viability, for example, may be tissue dependent, accounting for the growth of tumor in some parts of the body or, at a microscopic level, in specific areas of lymphoid tissue. To a degree, tissue-dependent survival is lymphoma subtype specific, such that each lymphoma is associated with a different, although overlapping pattern of tissue and organ distribution.

Broadly, lymphomas can be divided into B- and T-cell types according to the lineage they have arisen from and reflected in the predominant rearrangement of antigen receptor genes (T- or B-cell receptors). The uncommon NK cell–lineage lymphomas, encountered almost exclusively in pediatric lymphomas in anaplastic large cell lymphomas (ALCLs), is an exception to this, because, although more closely resembling T cells, they do not have rearranged antigen receptor genes. Lymphomas can also be classified according to whether their immunophenotype is of immature lymphoid or mature lymphoid origin. Because mature B- and T-cell lymphomas subserve different functions (respectively, the production of antibodies and the regulation, directly or indirectly, of other cell types, including the killing of cells expressing foreign antigens), B- and T-cell lymphomas have broadly different presentations, although there is considerable overlap. T-cell lymphomas, in general, are much more likely to involve the skin and even lung and muscle than are B-cell lymphomas, although both may frequently involve the nasopharynx, bone marrow, or central nervous system (CNS). In the case of precursor cell neoplasms, involvement of the thymus, the site of T-cell differentiation, is frequently present in immature T-cell neoplasms [e.g., lymphoblastic lymphoma (LL)]. This must be qualified, however, by the recognition that there are mature B-cell populations present in the thymus, such that some mature B-cell lymphomas may also present with thymic (mediastinal) enlargement.

The major childhood lymphomas that are discussed in this chapter include Burkitt's (BL), Burkitt's-like (BLL), and LBCLs, which fall on a continuous spectrum of histologic appearances but which all have a mature B-cell immunophenotype; LL, which is predominantly but not always of precursor T-cell origin; and ALCL and other peripheral T-cell lymphomas, all of which have a mature T-cell or null cell immunophenotype. The existence of a B-cell form of ALCL is controversial. These lymphomas can be grouped according to their histology and immunophenotype and, to a large degree, cytogenetic abnormalities. These subtypes differ with respect to their patterns of presentation, although there is overlap ( Table 24-1).

| Histologic category | Immunophenotype | Most common cytogenetic abnormalities[a] | Most common sites of presentation |
|---|---|---|---|
| Burkitt lymphoma, Burkitt's-like lymphoma, large B-cell lymphoma | B cell | t(8;14) and variants | Abdomen |
| Lymphoblastic lymphoma | Pre-T | Many | Thorax |
| | Pre-B cell | Many | Lymph nodes, bone |
| Anaplastic large cell lymphoma | T cell or null (natural killer?) | t(2;5) and variants | Lymph nodes, skin, soft tissue, bone |
| Other peripheral T-cell lymphomas | T cell | Unknown | Variable |

[a]Not all tumors in each category contain one of the translocations shown.

**TABLE 24-1. SUMMARY OF HISTOLOGIC CATEGORIES (WORLD HEALTH ORGANIZATION CLASSIFICATION), IMMUNOPHENOTYPE, AND MAJOR CLINICAL FEATURES OF CHILDHOOD NON-HODGKIN'S LYMPHOMAS**

One final area that needs to be addressed in these introductory remarks is the relationship between leukemia and lymphoma. All lymphoid neoplasms may present with diffuse involvement of the bone marrow, which may be accompanied by lymphomatous masses in other parts of the body. An arbitrary criterion of greater than 25% neoplastic cells in the bone marrow has been required for a diagnosis of leukemia among pediatric oncologists, but there is no biologic significance to this criterion, nor has prognostic significance been established. It is important to recognize that within each lymphoma category, bone marrow involvement is a sign of extensive disease rather than an indication of a different disease. Use of the term *acute B-cell leukemia* is unhelpful in this regard because this designation includes several lymphoma categories and is not, therefore, a precise diagnosis. The majority of such cases in children is BL with diffuse marrow involvement and should be treated as such. A smaller proportion is diffuse LBCLs, which can be treated with the same type of regimen used for BL. The designation of *acute B-cell leukemia* should not be interpreted as indicating that such patients should be treated with regimens used for acute lymphoblastic leukemia (ALL).

## HISTORICAL PERSPECTIVE

Before our modern conception of lymphomas, based on a knowledge of the normal immune system and of the molecular changes associated with lymphoma cells, there was a great deal of confusion regarding the nature and origins of what we recognize today as lymphoid neoplasms. With no unifying principles, it is not surprising that a plethora of terms, originating from many different perspectives (e.g., Ki-1 lymphoma, LL, centroblastic lymphoma, large cell lymphoma, BL), and a number of different classification schemes, some of which used the same term for different tumors (e.g., LL), were developed. Recently, improved classification systems for lymphoid neoplasms have been published—the REAL classification [4] and its derivative, the World Health Organization (WHO) classification [5]—and it is to be hoped that these will be sufficiently widely used to lead to a uniform usage of terms, although for long the excellent Kiel classification system [6] has held sway in Europe and may continue to do so. Just as the lack of knowledge concerning the origins of lymphomas led to competing classification systems, so too, the evolution of therapy has been largely empirical and at times arbitrary, and present approaches to treatment have resulted from empirical observations made over the course of many years. Perhaps in part because lymphoid neoplasms are highly responsive to chemotherapy, however, the most widely used regimens in use today are very effective, with anticipated cure rates of 80% to 90%, such that radical alteration of treatment approaches may be considered unethical unless justified by potential for significant benefit, such as a reduction in toxicity, and examined in a research context. It is, perhaps, worthy of note that this state of affairs has developed within a period of approximately 50 years, before which children with lymphoma had an abysmally poor prognosis. Indeed, as late as the middle of the twentieth century, lymphoid neoplasms were poorly understood, reflecting the lack of knowledge of lymphocytes and the immune system in general.

The modern era can be considered as beginning in the 1950s in Kampala, Uganda, where Denis Burkitt, an Irish surgeon, was working. Burkitt [7] recognized the clinical characteristics of the lymphoma still referred to by his name (after an intermediate period when, in the United States at least, the term *small noncleaved cell lymphoma* was preferred), although he initially believed it to be a sarcoma. At the same time, O'Conor [8] reported that approximately one-half of all childhood tumors seen in Kampala were lymphomas. Subsequently, O'Conor and colleagues,[9] Dorfman,[10] Wright,[11] and others realized that a proportion of childhood lymphomas in Europe and the United States were histologically indistinguishable from BL as originally described in Africa, although at the time considerable debate occurred as to whether the characteristic jaw tumors as well as the absence of bone marrow involvement should be required for this diagnosis to be made. Burkitt and, notably, Clifford also made major contributions to the treatment of childhood lymphomas by identifying a rather large list of chemotherapy agents to which BL would respond, occasionally with long-term cures.

### Evolution of Classification Schemes

When BL was incorporated into the earlier Rappaport classification, [12] the major classification scheme then in use in the United States, it was classified as a subtype of undifferentiated lymphoma—that is, a lymphoma that, at a time when immunophenotyping was not yet possible, showed no evidence of differentiation toward either a lymphocyte or a histiocyte, the two major categories of the Rappaport scheme. Subsequently, it was recognized that almost all lymphomas composed of large cells, which Rappaport considered to be of histiocytic origin, were actually of lymphoid origin. In the Rappaport scheme, undifferentiated lymphomas were classified into either Burkitt's or non-Burkitt's types, according to the degree of cellular pleomorphism. [12] Lukes and Collins,[13] recognizing that some cells in normal germinal centers of lymphoid tissue were similar to those of BL (or “undifferentiated lymphoma”), proposed replacing the term *undifferentiated lymphoma* by the descriptive term *malignant lymphoma of small noncleaved follicular center cells*, also to be subdivided into BL and non-BL. By then the division of the immune system into humoral (B) and cellular (T) components was established, and investigators recognized that lymphomas could also be classified on this basis. Because Lukes and Collins considered the small noncleaved cell lymphomas to be derived from germinal center cells, similar to most of the NHLs available to them for study, they considered them to be of B-cell lineage, a conclusion consistent with the earlier demonstration of the expression of surface immunoglobulin on the surface of BL-derived cell lines,[14],[15] and [16] and subsequently, on BL cells derived from patients in a leukemic phase. [17] Lennert and colleagues,[18] the main proponents of the Kiel classification scheme and also, from the early 1970s, advocates of classifying lymphomas on the basis of their presumed normal cell counterparts in the immune system (hence, into lymphomas of B- and T-cell origin), initially classified BL as a lymphoblastic B sarcoma (the term *sarcoma* being then used in European lymphoma classification

schemes to signify a highly aggressive tumor). Subsequently, they referred to it as a *malignant lymphoma of lymphoblastic type*, this category being subdivided primarily into BL and, following Lukes and Collins, lymphomas of convoluted cell types. Thus, LL, in the earlier German classification scheme, included the two major subtypes of NHL that we recognize today in children.

Lukes and Collins[13] described their malignant lymphoma of convoluted lymphocytes more than 16 years after the original description of BL. They suggested that this new entity was likely to be of T-cell origin and commented on the frequent involvement of the thymus and the higher frequency of the tumor in children and adolescents. Shortly afterward, Nathwani and colleagues[19] distinguished LL from the Rappaport category of diffuse, poorly differentiated lymphoma and identified it with the Lukes and Collins convoluted T-cell lymphoma, although Nathwani et al. also realized that many otherwise morphologically identical tumors lacked nuclear convolutions. Other neoplasms included in the category of diffuse, poorly differentiated lymphomas proved to be predominantly of B-cell origin, occurred almost exclusively in adults, and had different clinical courses from that of LL. It soon became apparent that LLs, similar to undifferentiated lymphomas, had a predilection at the time of relapse for the bone marrow and CNS and that most patients developed recurrent disease at these sites—at least in North America. [20,21] LL has subsequently been shown to be indistinguishable morphologically from ALL but to be almost the mirror image immunophenotypically, being of precursor T-cell origin in approximately 85% and of precursor B-cell origin in the remainder of cases (over 85% of ALL is of precursor B-cell origin).

Childhood lymphomas of the large cell type have been recognized for many years, being referred to by Rappaport as histiocytic lymphomas, as mentioned above, because of their large size and abundant cytoplasm.[12,20,22,23] Subsequently, Lukes and Collins[13] divided them into immunoblastic lymphomas and lymphomas of large follicle center cells, most of which were believed to be of B-cell origin. In the Kiel classification, these lymphomas were referred to respectively as immunoblastic and centroblastic lymphomas, the latter being the term used in Europe for the large cells in lymphoid germinal follicles. [14] Clearly there was considerable agreement between the Kiel and the Lukes and Collins classification schemes, both of which, as pointed out already, were presciently based on the probable normal counterpart cells in the immune system. In North America, however, the Rappaport classification remained in use, leading to considerable (if only continuing) confusion regarding the classification of NHLs. The National Cancer Institute of the United States (NCI), therefore, sponsored the development, in the early 1980s of what was called a *Working Formulation for Clinical Usage* and, presumably to avoid offending authors of rival schemes, presented it as a means of “translating” from one system to another.[24] The major drawback of what was, in effect, simply a new classification scheme, was that it was based purely on histology, and although it included terms used by Lukes and Collins, such as *malignant lymphoma of small noncleaved cells*, it most closely resembled the Rappaport scheme. European pathologists consequently continued to use the Kiel classification for the most part, and although the Working Formulation was used until quite recently, its failure to go beyond histology, with the result that each of its categories included multiple NHL subtypes, led to increasing difficulties for both pathologists and clinicians as new entities were described using a combination of histology, immunophenotyping and, increasingly, cytogenetic or molecular characterization. One of these new entities was a large, anaplastic lymphoma (ALCL) that expressed high concentrations of the Ki-1 antigen, originally recognized on cultured Reed-Sternberg cells. [25] It is now known that approximately one-half of childhood large cell lymphomas belong to the category of ALCL. Additional new entities included mantle cell lymphoma and lymphomas of mucosal-associated lymphoid tissue (MALT), and eventually a number of pathologists recognized that the time had come to develop a new classification scheme that incorporated the latest available information regarding the immunology and molecular abnormalities of lymphoid neoplasia.

Accordingly, in April of 1993, 19 hematopathologists from Europe and the United States recategorized lymphomas, using all of the criteria possible, including histology, immunophenotype, genetic features (cytogenetic or molecular), and their major clinical presentations and course. This resulted in the REAL (revised European American lymphoma) classification,[4] which has subsequently been updated in conjunction with a committee of expert clinicians, as the WHO classification. [5] In these classifications, all NHLs were divided into B- or T-cell lymphomas, each of which was further subdivided into precursor (i.e., lymphoblastic) or peripheral subtypes. Although of great significance to the understanding and classification of lymphomas in general, this has not resulted in major differences to the classification of the pediatric NHLs.

**Evolution of Therapy**

The results of treatment of pediatric lymphomas before the 1970s were extremely poor, with 5-year survival ranging from 5% to 33% (overall, closer to 5%) in various series.[2] The only therapy that could lead to cure in approximately 20% of patients with localized disease (stage I) was irradiation. [2,26,27] The use of single cytotoxic agents only slightly enhanced the results with radiation therapy alone, and because it was still not widely recognized that a high proportion of childhood NHLs were histologically identical to the African tumor discovered by Burkitt, treatment approaches evolved separately in Africa [28] and the United States and Europe.[2]

In African BL, some remarkable cures were observed by Burkitt and others with a small number of doses (even one) of cyclophosphamide or other chemotherapeutic agents, although most patients cured by such limited therapy also had limited disease. [28,29 and 30] Some North American patients with limited BL also achieved long-term survival after treatment with cyclophosphamide as a single agent.[31] The success of the MOPP [mechlorethamine, vincristine (Oncovin), procarbazine, prednisone] combination drug regimen in Hodgkin's disease[32] heralded the modern era of combination chemotherapy, and application of a combination of cyclophosphamide, methotrexate (MTX), and vincristine, initially in African BL [28] and subsequently in U.S. patients[33] demonstrated the advantages of combinations of drugs over single-agent chemotherapy.

Meanwhile, the clinical and morphologic similarity of LL to ALL and the high frequency of bone marrow relapses in lymphoblastic and non-LLs was instrumental in the development of the concept that malignant NHLs in children represented variants of acute leukemia. This concept led pediatric oncologists to apply regimens designed for ALL to the treatment of children with NHL, even in the absence of bone marrow involvement. The most successful of the leukemia regimens was the $LSA_2L_2$ regimen used at the Memorial Sloan-Kettering Cancer Center in New York.[34] The success of this regimen, coupled with the demonstration at the NCI that regimens based on repeated doses of cyclophosphamide, MTX, vincristine, and prednisone were quite successful in the treatment of BL, [33] led to the need to determine which of the two main approaches—lymphoma therapy, derived from the combination used in BL, or leukemia therapy, based on the successful $LSA_2L_2$ regimen—was more appropriate for the treatment of childhood lymphomas and whether prognosis with each of these regimens depended on histology. The results of this now classic study by the Children's Cancer Study Group (CCSG) indicated that the four-drug COMP regimen [cyclophosphamide, vincristine (Oncovin), MTX, prednisone], using the same drugs as in the NCI regimens, although with somewhat different dosage and schedule, was superior to the ten-drug $LSA_2L_2$ regimen for what were then referred to as *small noncleaved cell lymphoma*, but inferior to the $LSA_2L_2$ regimen for LLs.[35] Large cell lymphomas, comprising, in retrospect, both ALCLs and diffuse B-cell lymphomas, had a similar prognosis with both regimens. It was concluded from this study that lymphoblastic leukemia is best treated with an ALL-type regimen (in this case $LSA_2L_2$) while BL is best treated with repeated cycles of a cyclophosphamide and MTX-containing regimen. This principle has persisted until the present day, although the original 18-month therapy duration for BL has been drastically shortened and intensified for high-risk patients, and treatment results also appear to have improved in LL, although the optimal therapy duration for LL remains uncertain.

The treatment regimens in Africa did not include radiation therapy, because it was unavailable in Uganda. However, Swedish investigators working in Nairobi had reported very poor local control of BL with irradiation. [36] Despite this, the cure of a small number of patients with localized NHL using irradiation in the United States and Europe[37] led to the continued use of radiation directed to as many sites of disease as possible, as was practiced in the CCSG study referred to above. This tradition was slow to disappear but has at last been abandoned because of evidence from a randomized trial, and subsequent treatment of many more patients without radiation, showing that the latter modality increases toxicity without providing therapeutic advantage in patients with limited disease (treated, of course, with effective drug combinations).[38,39] In patients with advanced disease, excellent disease-free survival rates have also been obtained using chemotherapy alone (better, in fact, than those achieved when local radiation was used with less intensive chemotherapy), [26] and in many such patients, it would not be feasible anyway to irradiate all sites of disease.

Complete surgical resection of abdominal tumor was originally shown in African BL[40] to be associated with excellent survival rates if followed immediately by chemotherapy, although relapse remained relatively frequent. In North America and Europe, however, excellent results using chemotherapy alone, even in patients with advanced disease, have been achieved. This, coupled to evidence that the main determinant of outcome is tumor bulk rather than the surgery itself (at least in the United States and Europe), has led to surgical debulking no longer being recommended. [41,42] Nevertheless, a fraction of patients will continue to have complete surgical resection at the time of diagnosis—those, for example, who present with an acute abdomen, who generally have very limited tumor volumes that cause major symptoms because of intussusception or intestinal obstruction. Resection of all tumor is frequently necessary to deal with the acute problem. Such patients do very well,[39] but almost certainly, given their small tumor volume, would have done just as well had it been possible to treat them with combination chemotherapy alone, so that this observation cannot be said to support surgical resection as an elective procedure. It must be said, however, that in countries with limited resources, in which only minimal chemotherapy (e.g., cyclophosphamide as a single agent) can be given, surgical resection of tumor, as long as essentially all tumor can be resected without mutilation, may remain advantageous.

Since the classical studies of the mid-1970s, steady progress in the achievement of improved cure rates in all pediatric NHLs has been achieved. In B-cell

lymphomas, including BL and diffuse LBCL, and probably also in ALCL, this has resulted largely from the addition of high dose S phase agents along with additional drugs such as ifosfamide and etoposide to the therapy regimens of patients with more extensive disease. In LL, this has been associated with the gradual improvement of the regimens used for the treatment of ALL, particularly more intensive induction and consolidation (reinduction) regimens as well, perhaps, as the inclusion of relatively high dose asparaginase in these early treatment cycles.

## EPIDEMIOLOGY AND PATHOGENESIS

Lymphomas (Hodgkin's and NHLs) constitute 10% to 15% of all childhood cancers in the more developed countries; they are third in relative frequency after acute leukemias and brain tumors. In the United States, according to the NCI's Surveillance, Epidemiology and End Results program, 1.7% of all new cases of NHL occurring between 1992 and 1996 were in patients younger than 20 years old, and the average annual incidence rate for this age group was 1.0 per 100,000. [43] In 1996, 15.4% of all newly diagnosed cancers in persons younger than 20 years were lymphomas, and 6.4% were NHLs. Unlike Hodgkin's disease, which has a bimodal incidence curve, the incidence of NHL increases steadily with age throughout life, although the age-specific incidence of various subcategories varies markedly. The incidence of NHL in male children is approximately twice as high as that in female children. During the period 1973 to 1996, there was a 35% increase in the incidence of NHL in patients younger than 20 years but an increase of only 4.8% in those younger than 14 years. Although these figures are not greatly different from those in most European countries, the incidence and relative frequency of NHL varies considerably in different world regions ( Table 24-2): the incidence of BL varies from less than 1 to 36 per million children aged 0 to 14 years, whereas that of other NHLs ranges from 2.5 to 15.4 per million. [44]

| | Burkitt's lymphoma | | | Non-Hodgkin's lymphoma | | |
|---|---|---|---|---|---|---|
| | Male | Female | Total | Male | Female | Total |
| Uganda (Kampala) | 48.5 | 25.2 | 36.1 | 11.0 | 10.4 | 10.7 |
| Nigeria (Ibadan) | 24.1 | 11.9 | 18 | 2.1 | 1.5 | 2.0 |
| Israel (non-Jews) | 11.7 | 5.3 | 8.6 | 10.9 | 5.8 | 8.4 |
| Kuwait (Kuwaiti) | 7.2 | 7.4 | 7.3 | 10.3 | 2.7 | 6.7 |
| Brazil (Goiânia) | 7.7 | 4.6 | 6.2 | 4.8 | 2.1 | 3.5 |
| Israel (Jews) | 8.2 | 3.2 | 5.8 | 5.9 | 4.2 | 5.1 |
| France | 7.3 | 1.9 | 4.7 | 5.3 | 3.1 | 4.2 |
| Switzerland | 5.7 | 1.6 | 3.7 | 9.2 | 2.2 | 5.8 |
| Uruguay | 5.1 | 2.5 | 3.8 | 6.4 | 1.4 | 3.9 |
| United States (SEER), white | 4.1 | 0.7 | 2.5 | 7.6 | 3.5 | 5.6 |
| Zimbabwe (Harare) | 5.0 | — | 2.4 | 8.1 | 1.1 | 4.5 |
| Australia | 2.7 | 0.9 | 1.8 | 8.4 | 3.7 | 6.1 |
| Mali (Bamako) | 2.0 | 1.4 | 1.7 | 13.1 | 4.3 | 8.9 |
| Germany (former German Democratic Republic) | 2.3 | 0.3 | 1.4 | 7.8 | 3.0 | 7.3 |
| Algeria (Setif) | 1.1 | 1.6 | 1.3 | 8.1 | 5.1 | 6.9 |
| United States (SEER), black | 0.9 | 0.3 | 0.6 | 5.1 | 3.7 | 4.5 |
| India (Bombay) | 0.8 | 0.3 | 0.5 | 4.6 | 2.0 | 3.3 |
| United Kingdom, England, and Wales | 0.7 | 0.2 | 0.5 | 7.8 | 3.4 | 5.7 |

SEER, Surveillance, Epidemiology, and End Results program.
From Parkin DM, Kramárová E, Draper GJ, et al., eds. International incidence of childhood cancer, vol. II. IARC Scientific Publications, No. 144, with permission.

**TABLE 24-2. AGE-STANDARDIZED (0 TO 14 YEARS) INCIDENCE RATES PER MILLION OF BURKITT'S LYMPHOMA AND OTHER NON-HODGKIN'S LYMPHOMAS IN SELECTED REGISTRIES REPORTING TO THE INTERNATIONAL AGENCY FOR RESEARCH IN CANCER, LYON, FRANCE**

The incidence of NHL is much lower in children compared with adults, and it seems likely that age-related differences in the frequency of various histologic categories in children versus adults reflects, at least in part, the cellular composition of the immune system at different ages. Children and adults, however, also differ greatly in the type and duration of exposure to various environmental agents, and cell populations at risk as well as environmental exposures are likely to account for the differences in the patterns of NHLs at different ages.

### Geographic Differences in the Incidence of Lymphoid Neoplasms in Children

Just as the incidence of NHL varies markedly from country to country, [44] so does the relative frequency. In equatorial Africa, for example, approximately 50% of childhood cancers are lymphomas, [28] with this markedly increased frequency resulting from the very high incidence of BL in this region coupled to the low incidence of ALL. LL and large cell lymphomas also occur in equatorial Africa, probably with a similar incidence to that in the more developed countries, although accurate figures are unavailable and little is known of their biology. In Europe and the United States approximately one-third of childhood lymphomas are LL, one-half are BL or BLL, approximately 15% are large cell lymphomas (50% LBCL and 50% ALCL), and the remainder are unclassified.

### Burkitt's Lymphoma

There are a number of differences between equatorial African BL, often referred to as *endemic* because of the relatively high incidence of BL in this region, and BL in other parts of the world, frequently referred to as *sporadic* (Table 24-3). The term *sporadic* originally referred to BL in the United States and Europe and may not be appropriately used in other regions, in which the incidence, sometimes the pattern of presentation, and the proportion of tumors associated with Epstein-Barr virus (EBV) (see below) are between that of equatorial Africa and the United States and Europe. The reason(s) for these differences in incidence is unknown, although variability in the environment is the most likely explanation, perhaps resulting in differences in the cell type that undergoes transformation or in the set of genetic abnormalities present in the tumor cells. African BL was originally recognized because of the high incidence of jaw tumors, but this is not a site of predilection in patients outside Africa, although abdominal involvement is frequent in both endemic and sporadic varieties. One of Burkitt's [45] major contributions was to demonstrate the remarkable geographic distribution of the endemic tumor in equatorial Africa and to show that the apparent limits of its distribution were climatically determined. This observation led to the hypothesis that a vectored virus could be of pathogenetic importance and prompted a search for associated viruses that resulted in the discovery of EBV. [46] Although 95% of all equatorial African tumors carry EBV genomes in their cells, this is true for only approximately 15% to 20% of North American tumors. [28,47] Moreover, this difference does not simply reflect exposure to EBV, because even patients with EBV-negative tumors generally have antibodies to EBV [28,44] and the ubiquity of EBV infection clearly indicates that EBV itself is not responsible for the characteristic geographic distribution of BL in Africa. One striking difference in the epidemiology of EBV in Africa versus the regions in which “sporadic” BL occurs, however, is the difference in the age at which EBV infection occurs. Almost the entire population has been infected by the age of 3 years in Africa, whereas in affluent nations, primary infection frequently occurs in late adolescence or early adulthood. [48] BL in developing countries other than Africa also tend to be more often EBV associated (40% to 80%), supporting the notion that perinatal infection with EBV and low socioeconomic status increases the likelihood of developing BL. [49,50] and [51]

| | Endemic form | Sporadic form |
|---|---|---|
| Average annual incidence (children <16 yr) | 10 per 100,000 | 0.2 per 100,000 |
| Occurrence | Climatically determined | Not climatically determined |
| Association with Epstein-Barr virus[a,b] | 95% | 15% |
| Chromosome 8 breakpoints[c] | 75% upstream of c-*myc* | More often within c-*myc*[d] |
| Common sites of tumor | Jaw, abdomen, orbit, paraspinal, CNS | Abdomen, bone marrow, serous membranes |

CNS, central nervous system.
[a]Depending on world region.
[b]Presence of Epstein-Barr viral DNA in tumor cells.
[c]Applies to t(8;14) chromosomal translocations; in variant translocations breakpoints are invariably downstream of c-*myc*.

**TABLE 24-3. DIFFERENCES BETWEEN ENDEMIC AND SPORADIC BURKITT'S LYMPHOMA**

If we accept a role for EBV in the pathogenesis of BL—the International Agency for Research on Cancer considers that there is sufficient supportive evidence to draw this conclusion [52]—it remains still to be determined whether EBV infection simply predisposes to the development of African BL (and presumably those sporadic tumors that contain EBV sequences) or whether EBV is an essential component of pathogenesis. EBV's ability to infect and cause proliferation of B cells *in vitro* certainly accounts for its infectivity and persistence in humans. *In vivo*, this ability of EBV to “transform” B cells could result in an increase, whether transient or permanent, in the size of certain B- or pre-B–cell populations, with a consequent increased risk of the development of genetic abnormalities relevant to the genesis of BL in such cells, and this possibility, for long, was the leading hypothesis to account for EBV's association with BL. As more is learned of the functions of EBV genes,

however, the possibility that the expression of one or more of them in the tumor cells, or in precursor tumor cells, is relevant to pathogenesis, must be considered. Even during acute malaria, which is known to decrease T-cell mediated immunity directed toward EBV-infected cells and, consequently, to increase the fraction of circulating EBV-containing B lymphocytes and presumably the body burden of EBV-containing cells, [53,54 and 55] only a small fraction of the B cells present in the body are infected by EBV. Thus, the increased risk of developing BL based solely on an EBV-induced increase in the pool of B cells in which appropriate genetic changes can occur would seem to be small. Moreover, given the very small percentage of B cells infected by EBV, unless EBV has some kind of direct role, EBV association would be a very uncommon event.

Of course, if EBV does or can have a direct role in pathogenesis, the effect, in equatorial Africa at least, of the immunosuppressive effect of malaria (which is holoendemic in the regions of high incidence of BL in equatorial Africa), could be critical to EBV's potential pathogenetic role—increasing the risk of its development by a factor equivalent to the increased number of EBV-containing B cells, or at least, to the increased number of EBV-infected B cells capable of sustaining a relevant genetic lesion (it is highly unlikely, for example, that resting B cells would be susceptible to chromosomal translocation). Malaria-induced B-cell hyperplasia may even predispose to chromosomal translocation. Indeed, the close relationship between the distribution of malaria and that of BL in equatorial Africa is likely to account for the geographic distribution of this tumor within the African continent. More definitive conclusions are likely only to be reached by the identification of the specific molecular pathways through which EBV genes may act. Increasing information relating to the roles of the genes expressed by EBV in the latent phase of infection—that is, in the absence of virus production (which itself is incompatible with neoplasia because it results in cell lysis)—has at least identified several ways in which viral genes could contribute to pathogenesis. Of particular relevance to such considerations is the finding that one EBV protein, EBNA-1, is expressed in the vast majority of BL cells, so that if EBV does play a direct role in pathogenesis or, at least, in maintenance of the transformed phenotype, it could be via EBNA-1. This possibility is supported by the demonstration that EBNA-1 transgenic mice develop B-cell lymphomas. [56] Recently, the description of polymorphic variants of EBNA-1 and the demonstration that the most prevalent subtype is rarely associated with BL has provided further evidence that EBNA-1 may contribute to pathogenesis, although this story is still unfolding. [57] The negative effect of other EBV latent genes (EBNA-2 and LMP-1), both involved in the transformation of B lymphocytes, suggests that the EBNA-1–only pattern of gene expression in BL is a powerful clue to its role in BL. [58] Finally, the occurrence of familial BL in Tanzania provides evidence that inherited genetic abnormalities play a role, at least in some cases, in the pathogenesis of BL. [59]

### Other Non-Hodgkin's Lymphomas

Little epidemiologic information is available with regard to the other varieties of childhood NHL, but there is no doubt that the relative frequency of BL and LL varies markedly in different world regions. [60,61 and 62] LL appears to be very uncommon, along with ALL, in children in equatorial Africa. [63] No virus association has been observed with LL, nor is there a single specific chromosomal abnormality associated with LL, which appears to be similar to T-cell ALL with respect to its genetic lesions (see Chapter 17). Even though LLs are predominantly of T-cell origin, a small fraction have pre–B-cell characteristics. It seems likely that LL encompasses several etiologically distinct diseases. In Southeast Asia, virus-associated hemophagocytic syndrome (usually EBV) is associated with an increased risk of T-cell lymphoma development, and peripheral T-cell lymphomas represent a higher fraction of NHL in this world region. [64,65 and 66]

### Prelymphomatous States

Because lymphomas arise from the cells of the immune system, it is not surprising that disorders associated with abnormal regulation of lymphocyte proliferation and function are associated with an increased incidence of NHL. EBV also appears to be important in the pathogenesis of lymphomas in immunodeficiency states because the cells of a majority of these lymphomas and lymphoproliferative syndromes contain multiple EBV genomes. One possible explanation is that defective T-cell regulation permits an increase in the development and expansion of EBV-infected clones of B cells that would normally be tightly controlled but that, due to the influence of viral genes, have a selective advantage over uninfected cells. Such cell clones would then be at increased risk for developing genetic changes capable of causing neoplasia. The topic of lymphomas in immunosuppressed individuals is discussed in Chapter 25. Recently, a new syndrome known as *autoimmune lymphoproliferative syndrome* associated with mutations in the Fas (CD95) receptor was described, in which splenomegaly, lymphadenopathy, and autoimmune phenomena presumably occur as a consequence of defective regulation of T-cell activation because of impaired apoptosis. [67] Such patients have also been shown to be prone to the development of NHL of a variety of subtypes. [68]

### Drugs and Radiation as Predisposing Factors

There is little evidence that radiation is an important predisposing factor for lymphomas. In atomic bomb survivors, there appeared to be slightly increased prevalence rates of lymphoma in individuals who received more than 200 rads in Nagasaki and those exposed to more than 100 rads in Hiroshima, but there was no significant trend for increased incidence with increased dose during the period 1950 to 1978. [69] The relative risk during this same period for individuals exposed to greater than 200 rads was 1.6. The majority of the lymphomas in the exposed groups was NHLs of large cell type.

Although a four- to fivefold increased risk of lymphoma development has been observed in U.S. radiologists and uranium workers exposed during the 1950s and a twofold increase in the risk of lymphoma development observed for patients irradiated for ankylosing spondylitis for at least 6 years, there is little evidence for a lymphomagenic effect of the once-practiced thymus gland irradiation in children.

Patients with Hodgkin's disease treated with combined modality therapy are at increased risk to develop NHL—perhaps 4% to 5% of all patients so treated will develop NHL lymphoma within 10 years. [70,71] The precise risk for children with Hodgkin's disease treated with either chemotherapy alone or combined-modality therapy has not been determined because of small numbers of treated patients compared to adults. Apart from immunosuppressive drugs used in transplant recipients, information regarding the capacity of other drugs to induce lymphoma is limited. Hydantoin derivatives are known to cause pseudolymphomas that regress with cessation of therapy, and there are reports of the development of lymphomas in long-term recipients of these drugs. [72,73] However, no accurate figures are available regarding the size of this risk, but in view of the widespread use of these compounds, it is probably very low. [74] Various organic solvents, including dioxins, benzene, the herbicide 2,4D, and hair dyes have been incriminated in the etiology of NHLs, but there is no evidence that they represent significant factors in the occurrence of childhood lymphomas. [75,76 and 77]

## HISTOPATHOLOGY AND IMMUNOPHENOTYPE OF CHILDHOOD NON-HODGKIN'S LYMPHOMA

### Burkitt's and Burkitt's-Like Lymphomas

BL cells (Fig. 24-1) have a high nuclear to cytoplasmic ratio. The nucleus is round or oval and has an “open” nuclear chromatin pattern (i.e., giving the appearance of being able to see through the network of chromatin), a feature that contrasts with the appearance of the chromatin in LL. There are multiple (usually two to five), readily discernible nucleoli. Occasional cells may have only a single central nucleolus, but if such cells are frequent, many pathologists would diagnose BLL ( Fig. 24-2). The rim of cytoplasm is very basophilic (staining intensely with methyl green pyronine) because of the high RNA content and usually contains lipid vacuoles (which stain with lipid stains such as oil red O). Macrophages (so-called tingible body macrophages) are frequently interspersed among the tumor cells. These contain nuclear debris, probably ingested from tumor cells that have undergone apoptosis, and give rise to the oft-quoted “starry sky” appearance. This pattern is not pathognomonic of BL or BLL and may be seen in any rapidly proliferating tumor. BL and BLL differ with respect to the uniformity of the tumor cells. BL cells are, at least in histologic preparations, rather regular in size and shape, whereas BLL cells are more pleomorphic, with some cells being indistinguishable from BL cells, some indistingushable from large cell lymphoma cells, and some intermediate in appearance. As might be anticipated, the dividing line between these tumors cannot be made reproducibly. Indeed, in the new WHO classification, it is recognized that the histologic entity of BLL includes tumors that could well be classified as LBCLs, and with respect to biologic characteristics, may be closer to this entity than to BL, particularly in adults. Because such tumors, however, behave clinically as BL—that is, are rapidly progressive tumors and respond well to therapy designed for BL—it would be a mistake to eliminate this entity. On the other hand, some BLLs, particularly in children, contain the nonrandom chromosomal translocations that are observed in BL; it is likely that BLL also includes at least some classic BLs. These observations suggest that BLL may not be a biologic entity at all but is composed of a mixture of BL and a rapidly proliferative variant of LBCL, the relative proportions of each entity varying in children and adults (presumably because LBCL has a much higher incidence in adults). Until more definitive guidelines can be established, according to the new WHO classification, a diagnosis of BLL will only be made when a tumor with an appropriate histologic appearance also has a high proliferative potential, as evidenced by very high Ki-67 or MIB-1 positivity (99%). [5]

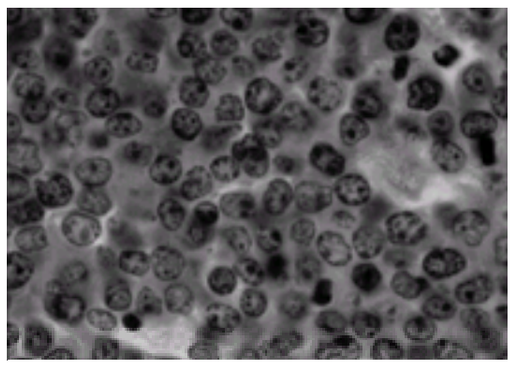


**FIGURE 24-1.** Histologic appearance of Burkitt's lymphoma. (From E.S. Jaffe. Histopathology and immunopathology. In Magrath I, ed. The non-Hodgkin's lymphomas, 2nd ed. London: Arnold, 1997:79–108, with permission.)

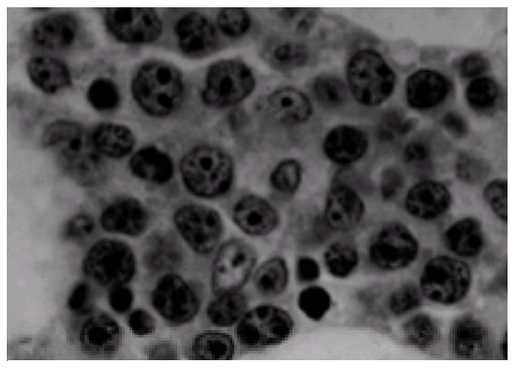


**FIGURE 24-2.** Histologic appearance of Burkitt's-like lymphoma. (From E.S. Jaffe. Histopathology and immunopathology. In Magrath I, ed. The non-Hodgkin's lymphomas, 2nd ed. London: Arnold, 1997:79–108, with permission.)

BL has the phenotype of a germinal center cell, expressing surface immunoglobulin (SIg), which, in more than 90% of cases, is IgM, associated with either kappa or lambda light chains. At a molecular level, the immunoglobulin (but not T-cell receptor) genes are always rearranged [4,5] and contain hypervariable mutations,[78,79] a finding that, in normal cells at least, is believed to indicate that the cell has entered the germinal center, the anatomical location at which hypervariable mutation and most class switching takes place. Other B-cell–associated antigens are expressed in BL, including CD19, CD20, CD22, CD79a, and CD77. [4,5] CD10, which is present on both lymphocyte precursor cells and germinal center cells, is also positive, as is CD38, another antigen expressed on germinal center cells, although terminal deoxynucleotidyl transferase (TdT) is negative. The immunophenotype of BLL is more variable, and in view of the diagnostic variability, less well characterized than BL, although similar. Occasional tumors may lack SIg, more often in adults. In at least some cases this may result from the presence of two translocations involving the immunoglobulin heavy-chain locus 14q32—both t(8;14) and t(14;18). [80,81] Rare tumors with the immunophenotype of immature B cells, including the expression of TdT, but containing 8;14 translocations have also been described.

**Large B-Cell Lymphoma**

LBLC comprises a heterogeneous mixture of tumors, at least with regard to their molecular genetic characterization. The degree of heterogeneity is likely to be greater in adults than in children, although little information on the genetics of LBCL in children has been published. There is some variation in the histology of LBCL, but attempts to subdivide LBCL reproducibly on the basis of histology have largely been unsuccessful. In adults, the separation of LBCL into centroblastic and immunoblastic subcategories may have prognostic implications. [82] In children, there is no evidence that this is the case. There is little doubt, however, that there are subcategories of LBCL. Those that present with a mediastinal mass, for example, appear to be the malignant counterpart of the B-cell population that is present in the thymus. It is also probable that at least some large cell lymphomas in children will prove to be similar or identical to LBCL in adults at a genetic level, but whether there is a subcategory of LBCL that occurs predominantly in children is unknown. In adults, LBCL accounts for the vast majority of rapidly proliferative B-cell lymphomas, and a number of distinct genetic lesions have been identified. The most frequent are chromosomal translocations involving the *bcl*-6 or *bcl*-2 genes. Approximately 5% to 10% have 8;14 or variant translocations, [83] suggesting that lymphomas that are biologically very closely related to BL can sometimes fall morphologically into the category of LBCL. In children, it seems likely that a higher percentage of LBCLs will prove to be of this type, because BL is the predominant childhood B-cell lymphoma and the morphological borders between BL and LBCL are not sharp. B-cell lymphomas (primarily LBCL, but sometimes BL) that arise in patients with underlying immunodeficiency may also differ biologically from their counterparts in nonimmunosuppressed children. LBCL, for example, is frequently associated with EBV in this setting.

LBCLs are composed of cells with nuclei generally larger than those of macrophages, which can usually be found among the tumor cells, and perhaps twice the size of a small lymphocyte nucleus (Fig. 24-3). The cytoplasm is basophilic, and nucleus vesicular with prominent nucleoli. The range of appearances of the tumor cells is variable, and most tumors comprise a mixture of cells that more or less closely resemble the centroblasts of germinal centers, immunoblasts, and less often multilobulated cells or even cells indistinguishable from those of T-cell ALCL, which are described below. Sometimes one or another of these populations predominates, hence the attempt by pathologists to subdivide these tumors. LBCLs have rearrangements of immunoglobulin genes, both heavy and light chain, but do not have rearrangements of T-cell receptor genes. They express B-cell associated antigens, including CD19, CD20, CD22, CD38, and CD79a. CD10 is occasionally expressed, but TdT is negative. SIg may be absent in up to one-third of these tumors [4,5] and is generally absent from LBCL of the mediastinum. It seems probable that most LBCLs originate in germinal centers or at least have a phenotype consistent with germinal center cells. The not infrequent lack of SIg is then consistent with the observation that a proportion of large, rapidly proliferating cells in normal germinal centers cells fails to express SIg. [84,85] It has been proposed that the temporary absence of SIg occurs during the process of somatic mutation of immunoglobulin gene variable regions. Immunoglobulins containing hypervariable region mutations are subsequently expressed on the cell surface, and cells bearing immunoglobulins with the highest affinity for antigen are selected by virtue of the greater likelihood that they will bind and be activated by antigen. [84,85 and 86] This process, which can involve multiple transitions between resting centrocytes and dividing centroblasts in normal germinal centers, each cycle associated with the production of additional mutations, is entirely consistent with the observation that LBCLs, similarly, carry multiple mutations (more than in BL) in the hypervariable regions of their immunoglobulin genes. [87] Indeed, the expression of CD38 and CD10 is also consistent with a germinal center cell phenotype of these neoplasms. Some tumors do appear to have a more mature phenotype in which plasma cell antigens, such as PCA-1, are expressed, and such cells may fail to express other B cell antigens such as CD19, CD20, CD21, CD24, and SIg.

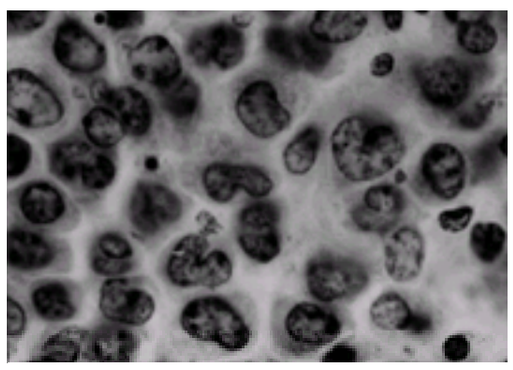


**FIGURE 24-3.** Histologic appearance of large B-cell lymphoma. (From E.S. Jaffe. Histopathology and immunopathology. In Magrath I, ed. The non-Hodgkin's

lymphomas, 2nd ed. London: Arnold, 1997:79–108, with permission.)

Whether there is an entity that should be referred to as anaplastic LBCL is still disputed, but a small number of LBCLs do appear to inappropriately express the p80 kinase. p80 is overexpressed in T-cell ALCL as a result of the formation of a fusion protein (NPM-ALK) by the 2;5 translocation. [88] In a small fraction of B-cell NHLs, p80 is overexpressed in the absence of this translocation. Such B-cell lymphomas may also express the CD30 antigen. Thus, there may be a B-cell equivalent to anaplastic T-cell lymphoma, although such tumors appear to be rare. Relatively low levels of expression of CD30 may also occur in the absence of other distinguishing features of ALCL—for example, on LBCL of the mediastinum.

LBCLs also contain various admixtures of normal cells, particularly macrophages and lymphocytes. On occasion, these populations can be sufficiently prominent (doubtless as a consequence of the production of relevant cytokines) to lead to nomenclature such as *T-cell rich B-cell lymphoma*. This entity is rare in children [89] and appears to be closely related to, and maybe coexistent with, a subtype of Hodgkin's disease known as *nodular lymphocyte predominant Hodgkin's disease*.

LBCL of primary mediastinal (thymic) origin is similar in appearance to other LBCLs, although the cytoplasm is more often pale than basophilic, and Reed-Sternberg–like cells may be present. There is often fine sclerosis within the tumor that can give the appearance of creating compartments.

**Anaplastic Large Cell Lymphoma**

ALCL is a relatively recently recognized entity [25] that was clearly misdiagnosed in the past as histiocytosis, carcinoma, sarcoma, or melanoma. The tumor has been given various and not necessarily synonymous labels, including Ki-1 lymphoma, because of its discovery through the use of this anti-CD30 antibody. [90] The term ALCL is based on the histologic characteristics of this entity. A number of subtypes have been defined on the basis of characteristic histologic features (e.g., lymphohistiocytic). [91] There remain rather imprecise borders between ALCL and Hodgkin's disease and ALCL and other peripheral T-cell lymphomas. [4,5,91] There can also be difficulties in differentiating between ALCL and the skin disease known as *lymphomatoid papulosis*. [91] That these two are related is clearly demonstrated by the transformation of some cases of lymphomatoid papulosis into ALCL. [92] In adults, ALCL can be secondary to a preexisting disease such as Hodgkin's disease or indolent peripheral T-cell lymphomas, particularly cutaneous T-cell lymphomas, but such secondary forms of ALCL are essentially unknown in children.

The hallmark of ALCL is the presence of anaplastic cells—that is, large pleomorphic cells containing horseshoe-shaped or multiple nuclei, with prominent, usually multiple, nucleoli (Fig. 24-4). Some of the cells may resemble Reed-Sternberg cells. The cells tend to be cohesive and to preferentially involve the sinuses of lymph nodes. Admixtures of normal cells, particularly granulocytes and macrophages, are present. In addition to CD30, ALCLs generally express the epithelial membrane antigen, EMA, which may lead to an erroneous diagnosis of carcinoma. They may or may not express CD45, CD15, or other T-cell–associated antigens. [4,5] A high fraction of ALCLs have a clonal rearrangement of T-cell receptor genes, usually Tb associated with biallelic deletion of Td, indicating Ta rearrangement, but some of these tumors appear to be of NK-cell origin and do not have rearranged T-cell receptor genes. [93,94] and [95] ALCL is uncommonly associated with EBV, particularly in children, [96] although some authors have reported significant rates of association. [97] ALCL is, however, nearly always EBV-associated in patients with underlying human immunodeficiency virus (HIV) infection.

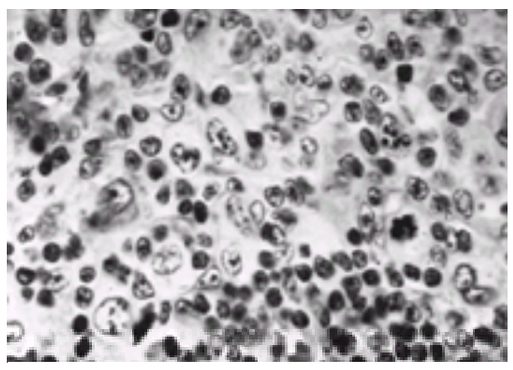


**FIGURE 24-4.** Histologic appearance of anaplastic large cell lymphoma. (Courtesy of Jeffrey Cossman.)

Sometimes the predominant malignant cells are not anaplastic but are small and relatively homogeneous. This subtype is known as the *small cell variant of ALCL*. In the lymphohistiocytic variant, the cells also tend to be smaller than the classic variety of ALCL, and there are copious admixtures of macrophages. A variant of ALCL exclusively involving the skin, which lacks the 2;5 translocation and EMA but expresses the cutaneous lymphocyte antigen CLA, has been described in adults, and although tumors that only involve the skin are occasionally seen in children, it is unclear whether this is the same entity as in adults. [98] Although most ALCLs contain a 2;5 translocation, it is not essential to the diagnosis. Recently, it was shown that inappropriate expression of ALK, the tyrosine kinase deregulated by the 2;5 translocation through the formation of the NMP-ALK fusion gene, can also be caused by other genetic abnormalities, including other translocations, [99,100] and [101] and it would seem probable that it is the ALK deregulation, rather than the mechanism of achieving this, that is the characteristic feature of systemic ALCL.

**Lymphoblastic Lymphoma**

LLs are indistinguishable histologically and cytologically from the lymphoblasts of ALL ( Fig. 24-5). The cells are usually quite uniform in appearance, with a high nuclear to cytoplasmic ratio—frequently even higher than that of BL, although there is variation in the quantity of cytoplasm from one tumor to another (similar to the L1 and L2 designations of ALL). A variable percentage of the cells may contain irregularly shaped or linear-patterned (“crow's foot”) nuclei. This appearance led to the term *convoluted T-cell lymphoma*. [13] The nuclear chromatin is finely stippled. Although multiple nucleoli are present, they are difficult to discern.

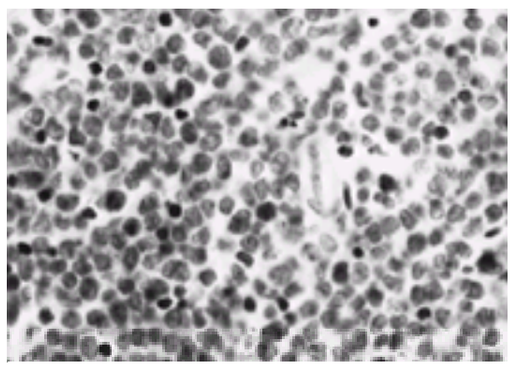


**FIGURE 24-5.** Histologic appearance of lymphoblastic lymphoma. (Courtesy of Jeffrey Cossman.)

LL, in addition to its distinctive histology, differs from all other lymphomas by virtue of the almost invariable presence of the enzyme TdT. [4,5,102] This can be demonstrated immunohistochemically, [103] and is a valuable means of confirming the diagnosis of a neoplasm derived from precursor lymphocytes. TdT participates in the generation of diversity in antigen receptor genes of both T and B cells by catalyzing the insertion of random nucleotides during the process of antigen receptor

gene rearrangements.[104,105] Its presence, therefore, indicates that the cell type is immature. Only a small percentage (some 5%) of LLs of the most mature stage of thymic differentiation fail to express TdT.[106,107] Many translocations probably occur around the time of B- and T-cell ontogeny, when precursor lymphocytes are rearranging their antigen receptor genes. It seems likely that such translocations involve the same enzymes that mediate antigen receptor gene rearrangement, because re-ligation of DNA ends produced by the immunoglobulin gene recombinases (RAGs), acting at specific recombination signal sequences, is mediated by nonhomologous end-joining enzymes such as Ku70 and Ku80 (which activate the large DNA–protein kinase subunit), XRCC4, and DNA ligase IV, all of which are involved in the repair of double-stranded DNA breaks in general.[108,109]

The presence of TdT is not diagnostic of a lymphoid neoplasm of precursor origin. Some nonlymphomatous tumors of childhood may express TdT, especially medulloblastoma.[110] Additional immunophenotypic characteristics are also essential in diagnosis. Mantle cell lymphoma, for example, has a blastic variant that closely resembles LL morphologically. Study of the expression of TdT, SIg and cyclin D1 (CD99), which is deregulated in mantle cell lymphoma, are valuable means of distinguishing these tumors, which respond very differently to therapy.[111]

The majority of LLs arises from T-cell precursors undergoing differentiation in the thymus.[112] They express T-cell markers corresponding to the stages of T-cell intrathymic differentiation, usually designated as early, intermediate, and late ( Fig. 24-6).[113,114] CD7 is a particularly valuable marker of the early T-cell lineage and is present on essentially all immature T-cell malignancies—that is, LLs and ALLs of T-cell type.[115,116] Additional antigens expressed at the surface of T-cell LLs predominantly reflect an intermediate (CD7, CD5, CD2, CD1, CD3±, CD4, and CD8) or late thymocyte phenotype (CD7, CD2, CD3, and either CD4 or CD8), although atypical patterns are quite frequently observed.[117,118,119 and 120] CD2 is usually present on LL cells, although it may not be expressed by the earliest cells of the T lineage. Such normal cells do, however, frequently express CALLA (CD10) and HLA-DR antigens, although these antigens are not commonly detected on LLs. CD3, a heterodimer that is expressed in concert with the antigen receptor itself to form the antigen receptor complex[120] may be present in the cytoplasm of immature T cells even though it is not expressed at the surface.[121] CD1 is only expressed on intermediate thymocytes, whereas CD4 and CD8 are expressed on the same cell in intermediate thymocytes but separately on more mature cells of helper and suppressor (or cytotoxic) phenotypes, respectively. All of the antigen receptor molecules are present in the cytoplasm before expression at the cell surface in concert with the CD3 protein.[122,123]

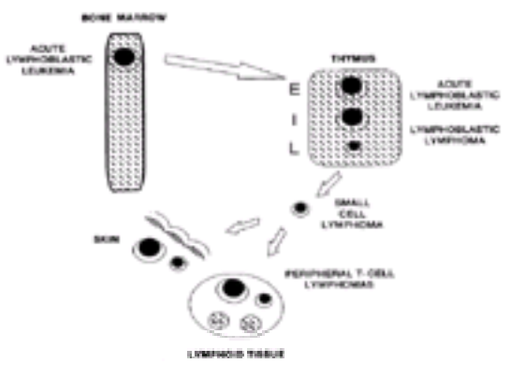



**FIGURE 24-6.** Cellular origins of T-cell lymphomas. T cells originate in the bone marrow and migrate to the thymus where they undergo differentiation. Intrathymic differentiation is arbitrarily divided into three main stages: early (E), intermediate (I), and late (L). Lymphoblastic lymphomas (LLs) predominantly arise from the intermediate and late stages, hence their frequent presentation as a thymic (mediastinal) mass. Bone marrow involvement may occur, however, whether the phenotype is early, intermediate, or late such that there is no clear distinction between T-cell acute lymphoblastic leukemia and LL apart from the arbitrarily chosen degree of bone marrow involvement (25%). T-cell lymphomas with a mature phenotype (peripheral T-cell lymphomas) may occur at any of the sites at which T cells are found—that is, in almost all organs and tissue of the body, but particularly in lymph nodes, and at surfaces prone to invasion by microorganisms or foreign bodies.

It is not at all clear that acute lymphocytic leukemia and LL differ in their clinical characteristics because they are neoplasms of different thymocyte stages. Leukemias tend to have a more immature thymocyte phenotype, but in a series of T cell ALLS from Egypt, the phenotype was predominantly that of intermediate (58.1%) or late thymocytes (16.1%).[124,125] Bone marrow and blood involvement is, therefore, an unreliable discriminator between early and late-stage T-cell lymphoblastic neoplasms. Furthermore, although the likelihood of a mediastinal mass is greatest in the intermediate group, and least in the most mature and immature groups,[107,118,126] mediastinal masses are frequently present in both T-cell LL and leukemia. In this circumstance, there appear to be no differences in the immunophenotype whether the disease is classified as lymphoblastic leukemia or LL.

T-cell LLs have clonal rearrangements of their T-cell receptor genes, which correlates with their degree of differentiation. The most immature neoplasms usually have a clonal rearrangement of a Td gene without rearrangement of other T-cell receptor genes.[120,122,127] Such tumors sometimes express Td transcripts. Rarely, in pre-T neoplasms, all of the T-cell receptor genes are in the germline configuration. T-cell neoplasms of intermediate maturity (cytoplasmic CD3) have rearrangements of Td, Tg, and Tb, and some may have biallelic deletion of Td, which indicates a rearrangement because the d locus lies within the a locus. The more mature lymphoblastic neoplasms (expressing surface CD3 with either CD4 or CD8) have Tg and Tb rearrangements with biallelic deletion of Td.[122,127,128] This pattern is similar to that of the peripheral T-cell lymphomas and is sometimes accompanied by expression of Ta/b transcripts.[120] Ten percent of T-cell lymphoblastic neoplasms have rearrangements of immunoglobulin receptor genes.[129,130 and 131]

Approximately 10% to 15% of LLs express the phenotype of pre-B cells as seen in ALL—CD19 and HLA-DR, usually with CD10 but without SIg.[132,133] Such tumors have a rearrangement of at least a heavy-chain gene, and may, similar to ALL, have a rearrangement of a light-chain gene. Of considerable interest is the pattern of rearrangement of T-cell receptor genes in such cases. Seventy percent to 100% have rearrangements or deletions of the Td gene, and only a slightly lower proportion, of Tg. Some 10% have rearrangements of Tb,[129] and some even have biallelic deletion of Td, indicating Ta rearrangement.[120,122,127,130] This could be due to an origin of these neoplasms from an uncommitted precursor cell. However, the lack of T-cell immunologic markers in such tumors argues against this. The alternative hypothesis is that rearrangement of antigen receptor genes occurs in these tumors because the cell type is one in which the recombinases that catalyze such events remain active for at least some time after malignant transformation.[122,130]

Recently, LL with a phenotype consistent with that of NK cells (including CD56 expression) was described.[134,135 and 136] The few cases so far reported appear to have a poor prognosis.

### Rare Childhood Non-Hodgkin's Lymphomas

Although the vast majority of childhood lymphomas can be categorized as BL, BLL, LBCL, ALCL, or their variants, tumors that occur commonly in adults are occasionally seen. These include marginal zone B-cell lymphomas,[137] although these are significantly more common in children with HIV infection,[137a] follicular lymphoma,[138,139,140,141 and 142] and peripheral T-cell lymphomas that do not fall into the category of ALCL.[139,143,144,145 and 146] Histology and immunopathology are similar to equivalent adult tumors, but it is likely that there are biologic differences. In childhood follicular lymphoma, for example, the disease tends to be localized, testicular involvement has been reported more frequently than would be expected, and the chromosomal translocation (14;18) present in adult follicular lymphomas is absent, at least in the testicular cases.[142,147] In patients described to date, the prognosis is good even with conservative management.[140,141] Peripheral T-cell lymphomas may be more common in some world regions, for example, Southeast Asia, where at least some arise in the context of a virus-associated hemophagocytic syndrome frequently caused by EBV.[64,65 and 66] Another rare disease, hepatosplenic g/d lymphoma, occurs predominantly in young adult males but rarely in children younger than 15 years.[148,149,150 and 151] To date, such patients have had a poor prognosis regardless of treatment.

## CYTOGENETICS AND MOLECULAR PATHOLOGY

In the NHLs of childhood, a number of nonrandom cytogenetic abnormalities, predominantly reciprocal chromosomal translocations, have been identified. Many such translocations involve an antigen receptor gene—either an immunoglobulin or a T-cell receptor gene, depending on the cell lineage—at one of the locations of the chromosomal breakpoints. There are good reasons to believe that the enzymatic apparatus needed for antigen receptor gene rearrangement may also mediate

chromosomal translocation.[152,153] Although the signal sequences required for the recombinases (RAG-1 and RAG-2) are generally absent from the sites of chromosomal recombination or, at best, show poor homology, these could well have been removed during the process of translocation. Furthermore, RAG genes are responsible only for cleaving the DNA at specific cleavage sites, whereas re-ligation is accomplished by nonhomologous recombinases. [154] Such enzymes seem very likely to be involved in mediating chromosomal translocations. An alternative possibility is that differences in chromatin structure that occur in the process of gene rearrangement[155] may provide a "fragile site" prone to breakage. In either event, it seems probable that immature lymphoid cells capable of antigen receptor gene rearrangement are particularly prone to translocations involving these genes and, further, that nuclear topography may be a factor in determining the most likely partner chromosomes in differentiating or proliferating cells. [156] Because a single genetic event is insufficient to induce neoplasia, it should not be assumed that cell differentiation is blocked at the time of the first genetic event. Cells may well undergo further differentiation during the accumulation of additional lesions—indeed, such differentiation may even be necessary to this process. This is consistent with the possibility that translocations occur at or close to the point in lymphoid differentiation at which immunoglobulin or T-cell receptor gene rearrangement is occurring, although the final phenotype is that of a more mature cell.

The translocations that occur in lymphoid neoplasia frequently involve genes whose products function as transcription factors, that is, proteins that regulate the expression of genes, or as proteins that modulate the activity of transcription factors, usually via direct binding to the transcription factors. Two major patterns can be identified: those in which one of the involved chromosomal regions is an antigen receptor locus (BCR or TCR) and another in which a fusion gene and fusion protein results from the translocation (Fig. 24-7). It is probable that the frequency with which genetic abnormalities result in the modulation or alteration of transcription factor function arises from the fact that such genes may have critical roles in the control of or interactions between entire molecular pathways relevant to cell proliferation or differentiation. In the first of the two major types of translocations involving transcription factors, abnormal expression of a transcription factor occurs because its regulation is usurped by the regulatory regions of the antigen receptor gene, which is expressed continuously in cells of the lymphoid lineage beyond the precursor stage. In the second type of translocation, a chimeric or fusion gene consisting of parts of two transcription factors is created. It appears that the chimeric protein is expressed as if it were the gene comprising the upstream element of the fusion gene, which contains the promoter and at least some of the regulatory elements of the gene. The functional properties of the chimeric protein (i.e., the specificity of DNA binding and genes regulated by the transcription factor) may be precisely those of the downstream fusion partner, or possibly different from either of the partners because of new properties conferred on the fusion protein.

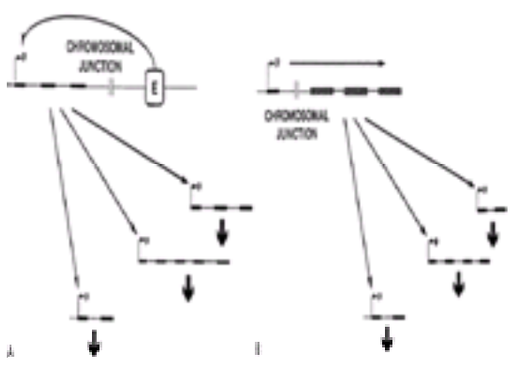



**FIGURE 24-7.** The major types of chromosomal translocation that have been described in leukemias and lymphomas. **A:** Deregulation by juxtaposition of a gene on one chromosome to an enhancer region (a regulatory element that can increase transcription from upstream or downstream promoters)—frequently one associated with an antigen receptor (T- or B-cell) locus—on a second chromosome. **B:** Fusion of two genes, each located on a different chromosome but in the same orientation. This fusion (chromosomal junction) leads to various effects—for example, the expression of one (i.e., the downstream component) as if it were the other (i.e., the upstream component, which includes a promoter), or enhanced or altered function due to alteration in protein binding properties, changes in phosphorylation, or other functions. A similar effect—that is, generation of a fusion protein—can be created by deletion of sequences between two genes. In this diagram, the genes are shown as transcription factors that influence the expression of other genes, but genes involved in other vital cellular functions, such as kinases (e.g., ALK, described in the text), or anti-apoptotic, genes may be involved in either type of translocation, with resultant important functional consequences. E is an enhancer, frequently derived from an antigen receptor locus, and P indicates promoters. The transcription factors and the genes they control are shown as lines (signifying intervening sequences, or introns) and boxes (signifying exons, which are present in the mature messenger RNA). Long arrows indicate positive transcriptional influences, either *cis* or *trans*. Broad arrows indicate expression of the genes controlled by the transcription factors.

Among other important functional consequences of translocations is the altered (usually overexpression) of a kinase—that is, a protein involved in the activation or deactivation of other proteins. Such kinases have a greater or lesser degree of specificity and can, in some ways, be considered to subserve a regulatory function at the protein level, which is the equivalent of that mediated by transcription factors at the level of messenger RNA (mRNA) expression. Finally, whether mediated by a translocation or by another type of genetic abnormality, altered proliferation or differentiation by itself is not enough. Because of the critical regulatory role that apoptotic pathways serve in the regulation of both of these essential components of the lives of cells, relevant apoptotic pathways must, in some way, be inactivated or at least prevented from causing the death of the potential or actual tumor cell because of the modifications brought about in its proliferation or differentiation. Indeed, because the latter are fundamental cellular activities, which are essential to the very existence of the organism, they are invariably connected to apoptotic pathways.[157,158,159 and 160] The processes of cell proliferation, differentiation, and survival are thus closely integrated, as is well illustrated by the example of c- *myc,* a gene that appears to be a central element in a broad range of cellular functions (not excluding metabolism) relevant to these processes. [3,162]

New microarray techniques that permit the examination of the expression of tens of thousands of genes simultaneously may lead to a new understanding of the consequences of the genetic abnormalities of neoplastic cells, because the consequences of all genetic lesions must ultimately be a modification of the pattern of gene expression.[162] Examination of protein expression patterns proteomics may provide even more information than does the presently more widely used mRNA expression microarray analyses. In addition to an understanding of pathogenesis, such techniques are likely to lead to more precise diagnosis as well as improved prediction of response to treatment. In some cases, it may be useful to define remission at a level beyond that of clinical and radiological examination. For this, polymerase chain reaction (PCR) is likely to be more useful than microarray analyses, because it is capable of identifying individual tumor cells among hundreds of thousands of malignant cells. Molecularly defined remission using PCR is already possible because several of the nonrandom chromosomal translocations or other genetic lesions associated with lymphoid neoplasia can be detected by this technique, providing a highly sensitive method for the detection of residual cells from the malignant clone (also detectable via identification of the clone at the level of BCR or TCR). Detection of the 14;18 translocation, in particular, has undergone and continues to undergo extensive evaluation with respect to the definition of molecular remission and its clinical utility. [163,164 and 165] This translocation is uncommonly associated with childhood NHL, although it may occur in some LBCLs or BLLs. Because of the possibility of identifying specific molecular changes through PCR, this technique may also prove to be a useful adjunct in diagnosis and risk assignment, while the genetic lesions themselves provide potential targets for new, highly specific therapeutic approaches.[166,167 and 168]

### Burkitt's Lymphoma

BL is associated with characteristic nonrandom, reciprocal chromosomal translocations that are predominantly between chromosomes 8 and 14 (some 80% of tumors),[169] and less often, between chromosomes 8 and 2 or 8 and 22.[170] The breakpoint on chromosome 8 coincides with the location of a proto-oncogene (c- *myc*, on band q24),[171,172 and 173] while the breakpoint on the other partner chromosome is in an immunoglobulin chain locus—either that of the heavy chains (chromosome 14, band q32) or that of one of the light chains (chromosome 22, band q11 or chromosome 2, band p11/p12, the loci of lambda and kappa genes, respectively). [173] In 8;14 translocations, the c- *myc* gene is translocated from chromosome 8 to the heavy chain locus on chromosome 14, whereas in the so-called variant translocations involving immunoglobulin light chains a part of the constant immunoglobulin locus is translocated to chromosome 8, distal to the c- *myc* gene. The common feature of all three translocations is the juxtaposition of the c- *myc* gene to immunoglobulin constant region sequences, whether of heavy- or light-chain origin ( Fig. 24-8). The expression of the c-*myc* gene, whose own regulatory regions are damaged or even deleted during the process of chromosomal translocation, becomes subordinate to the influence of the immunoglobulin gene regulatory sequences that now lie adjacent to it on the same chromosome. Because immunoglobulin genes are constantly expressed in B cells, the c-*myc* gene remains switched on, even when it should not be expressed. The Myc protein is known to be necessary for cellular proliferation,[174,175] and it is, therefore, highly likely that it is the inappropriate expression of Myc, occasioned by the translocation, that maintains the cell in a proliferative state. It is unlikely, however, that deregulation of c- *myc* alone is sufficient to cause BL, in spite of its broad range of functions relevant to cell growth and differentiation,[161,176] because its inappropriate expression (i.e., expression in the absence of appropriate signals induced by growth factors) will cause apoptosis. [3] Myc overexpression would, therefore, be expected to lead to cell death in the absence of additional genetic lesions within the Myc-regulated apoptotic pathways, such as

the Fas (CD95) pathway.[3,177] The myc-immunoglobulin translocation and its consequences are depicted in Figure 24-9. It has been postulated that the chromosomal translocation occurs in a proliferating immature B cell either during or shortly after the process of immunoglobulin gene rearrangement. [178] The possibility that the chromosomal translocation may occur in a germinal center cell cannot be ruled out, because some breakpoints are in switch regions, and it has recently been shown that immunoglobulin recombinases are expressed in germinal center B cells. [179,180] However, such cells clearly have an immature phenotype, and because the switch region is susceptible to chromosome breakage even in pro-B cells [181] and heavy-chain class switching has been described in pre-B cells, [182] these observations do not refute the likelihood that translocations occur in immature B cells. The hypothetical scenario in which the chromosomal translocation occurs in immature B cells is depicted in Figure 24-10.

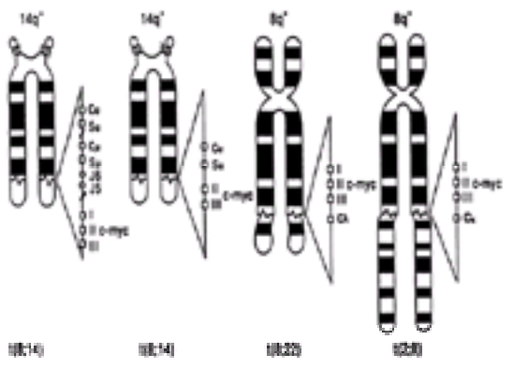


**FIGURE 24-8.** Chromosomal translocations occurring in Burkitt's lymphoma at cytogenetic and molecular levels. In each case, the c- *myc* gene is juxtaposed to an immunoglobulin heavy- or light-chain constant region. Two different types of t(8;14) are shown in which various gene segments of c- *myc* (I, II, III) and Ig heavy [Cμ, Sμ, J(no.), etc.] are shown in different relationships. See Figure 24-9 for further information. Cμ, μ constant region; J(no.), one of several J segments; Sμ, μ switch region; I, first exon of c-*myc*; II, second exon of c-myc; III, third exon of c-*myc*.

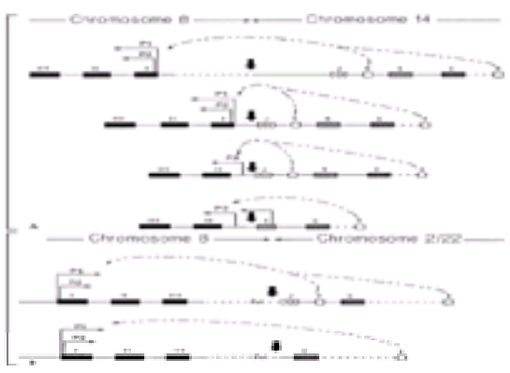



**FIGURE 24-9.** Molecular rearrangements that occur as a consequence of the 8:14 translocation. **A:** Juxtaposition of c-*myc* to the heavy-chain locus on chromosome 14. From the top down, it is seen that the breakpoint can vary in different tumors: upstream of c- *myc*, in the immediate 5' region, or in the first exon or intron. In all of these diagrams, the breakpoint on chromosome 14 is depicted in the J region. In the lowest diagram of **(A)**, the breakpoint is shown in the switch μ region of the immunoglobulin gene; it may also occur, although less commonly, in other switch regions of the heavy-chain locus. **B:** Juxtaposition of c-*myc* with a light-chain locus, as occurs in the variant translocations. The Pvt region was first described in mouse plasmocytoma as a cluster region for variant translocation breakpoints. Its significance remains uncertain. Exons are shown as boxes, either solid, hatched, or open.

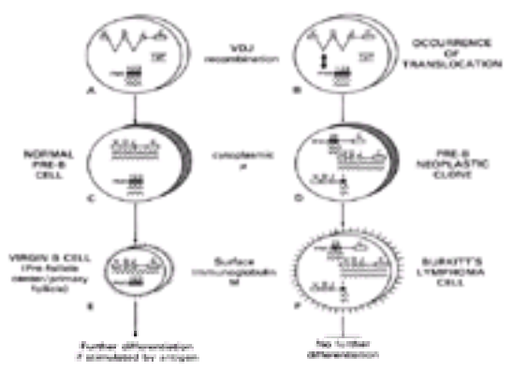



**FIGURE 24-10.** Hypothetical schema depicting the pathogenesis of Burkitt's lymphoma. On the left, the normal series of differentiation steps (including VDJ joining) for the earliest stages of B cells, resulting first in cytoplasmic μ chains **(C)** and then in surface immunoglobulin M (IgM) **(E)** are shown. The earliest cells expressing surface immunoglobulin M (virgin B cells) are resting cells, unlike their more immature counterparts, and they have switched off expression of c- *myc*. On the right, chromosomal translocation and deregulation of c-*myc* has occurred, with resultant indefinite proliferation of the cells containing the translocation. Normally, such deregulation of c-*myc* would result in diversion to an apoptotic pathway. Other genetic lesions are therefore necessary to permit the cell to survive as a rapidly proliferating lymphoma cell. TdT, terminal deoxynucleotidyl transferase; VDJC, variable, diversity, joining, and constant immunoglobulin gene regions; ∿∿, gene expression.

In BL the breakpoint location on chromosome 8 varies with the geographic origin of the tumor ( Fig. 24-11).[183,184 and 185] In endemic (equatorial African) lymphomas the breakpoint is, in most cases, some distance away from the c-*myc* gene. In this circumstance, however, there are mutations in the first exon of c-*myc*, which appear to contribute to the deregulation of the gene. In sporadic (North American) tumors the breakpoint is usually within the gene or in its immediate upstream flanking sequences, a region that is known to contain sequences involved in the regulation of c- *myc* expression, such that a variable but often considerable portion of the regulatory region of c-*myc* is separated from it.[173] In regions other than equatorial Africa and the United States and Europe, intermediate situations are found with respect to the fraction of tumors with breakpoints in various regions in and around c- *myc* on chromosome 8.[49]

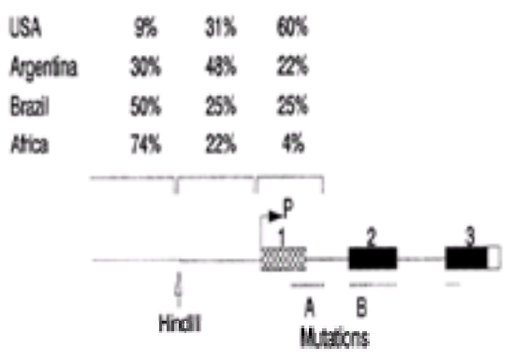



**FIGURE 24-11.** The proportions of Burkitt's lymphomas from different world regions with breakpoints in various regions (shown by square brackets) of chromosome 8

are shown in the table. The diagram underneath the table depicts exons 1 to 3 of the c- *myc* gene and indicates the locations of the breakpoint regions and mutations in regulatory (A) and coding (B) regions. Some tumors from Chile are included in the row labeled “Argentina.” HindIII is a restriction enzyme site that provides a useful marker upstream of the promoter, P.

In addition to the chromosomal translocations themselves, some of which lead to considerable structural changes in the *c-myc* gene, such as detachment of exon one from the coding region, associated point mutations also occur—both in the regulatory elements of c- *myc*, when these are intact (depending on the precise breakpoint location), and in the protein coding region. The former are doubtless associated with altered regulation of the expression of the gene, whereas the latter appear to alter either the half-life of the Myc protein or the ability of another protein, p107, to regulate its ability to function as a transcription factor. [185,186]

BL also frequently contains (in approximately 40% of tumors at presentation) p53 mutations, [187] and recently mutations in the Rb family of proteins, including p130 and p107, which may differ in sporadic versus endemic tumors have been reported. [188]

**Burkitt's-Like Lymphoma and Large B-Cell Lymphomas**

BLL, as previously discussed, is likely to comprise a mixture of BL and LBCL, and indeed, some of these tumors are associated with the same translocations that occur in BL and some with translocations that occur in a fraction of adult LBCLs. Some cases of BLL may also more closely resemble, immunophenotypically, a subset of LBCL than BL. [189] LBCL in adults is inhomogeneous with respect to the presence of cytogenetic or molecular changes, manifesting, in different tumors, various cytogenetic lesions, particularly 14;18 translocations and translocations involving *bcl*-6. [80,153] In LBCL in children, there are very limited data, although a recent report was consistent with the probability that at least some cases of LBCL in children are similar to those in adults. [189a] BLLs that contain both 8;14 and 14;18 translocations have also been described, and it has been suggested that possession of both translocations, at least in adults, is a poor prognostic sign. [190]

**Lymphoblastic Lymphomas**

Although nonrandom chromosomal abnormalities have been found in LLs, unlike BL, many different translocations can be found. Those that occur, as with many of those present in lymphoid neoplasia, frequently involve the BCR and TCR genes. In the case of T-cell LL, the Ta/d locus situated on chromosome 14q11 is frequently involved in, for example, 11;14, 1;14, 8;14, and 10;14 translocations. These findings strongly suggest that the rearrangement of genetic material occurred close to the time when rearrangements of T-cell receptor gene subunits normally occur. However, chromosomal translocations that do not involve the antigen receptor loci have also been described in T-cell LLs and leukemias.

Insights into the pathogenetic mechanisms relevant to T-cell lymphoblastic neoplasia have been gained by molecular cloning of the genes located at translocation breakpoints. In two cell lines that contain 8;14(q24;q11) translocations, for example, the Ta constant region has been shown to be translocated from its location in chromosome 14 (the breakpoint being in the J region of the Ta gene) to a point distal to c- *myc* on chromosome 8. [191,192 and 193] This type of translocation is analogous to the variant translocations observed in BL and, similarly, results in deregulation of c- *myc*. A particularly common molecular abnormality in T-cell lymphoid neoplasia is overexpression of the Tal-1 or Scl gene, as occurs in the 1;14 translocation, but resulting more often from a deletion on chromosome 1 that juxtaposes the *scl* or *tal*-1 gene, which is not normally expressed in lymphoid cells, to another gene, which is normally expressed, Sil-1. [192,194] There is evidence that the chromosomal deletion that results in this particular genetic rearrangement is also mediated by VDJ recombinases. [194] There can be little doubt that, as in B cells, the consequences of the translocations associated with neoplasia in T cells are to alter the differentiation and apoptotic patterns normally found in T-cell precursors. [195,196 and 197]

**Anaplastic Large Cell Lymphomas**

In children, the majority of ALCLs (approximately 80%) contain 2;5 translocations, [198] which despite a few reports to the contrary appear to be largely confined to ALCL lymphoma of the T-cell or null cell type. [88,198 and 199] This translocation does not appear to be associated with the rare subtype of ALCL (occurring predominantly in adults), which involves only the skin. The 2;5 translocation juxtaposes the tyrosine kinase ALK to a widely expressed gene known as *nucleophosmin* such that ALK is inappropriately expressed. [88,199] Recently a number of variant translocations that are also associated with inappropriate expression of ALK, but not with the fusion protein NMP-ALK have been described. [200,201,202,203 and 204] The NMP-ALK fusion protein is expressed in the nucleus, because NMP is a nucleoprotein, as well as in the cytoplasm, but in the variant translocations, which do not involve NMP on chromosome 5, ALK protein expression is confined to the cytoplasm. [204] At present, the mechanism whereby inappropriate expression of the ALK tyrosine kinase is relevant to pathogenesis is unknown. The ALK protein, however, whose xpression normally appears to be confined to the nervous system, has transforming properties *in vitro* and NMP-ALK can cause large cell lymphoma when transfected into murine bone marrow. [88]

## CLINICAL PRESENTATIONS

Patients with NHL present, for the most part, with a limited number of clinical syndromes, which correlate reasonably well with the histologic category, although there is significant overlap. Childhood lymphomas are much more often extranodal than are adult lymphomas, the most frequently involved sites being intraabdominal (B-cell lymphomas) and intrathoracic (precursor T-cell lymphomas). The involvement of some sites, for example, lung, skin or muscle, is sufficiently uncommon to raise questions about a diagnosis of lymphoma, although these sites may be involved in patients with ALCL [205] or in lymphomas arising in patients with HIV infection in whom atypical presentations are common. Although systemic symptoms including weight loss and fever may occur, these are relatively uncommon, except in ALCL or, in the case of weight loss, in circumstances in which there are mechanical reasons for poor food consumption.

**Burkitt's Lymphoma**

***Sporadic Burkitt's Lymphoma***

A large proportion of children with BL in the United States and Europe present with abdominal tumors that give rise to abdominal pain or swelling, sometimes with a symptom complex caused by intussusception, a change in bowel habits, nausea and vomiting, evidence of gastrointestinal bleeding or, rarely, intestinal perforation. [206,207 and 208] Presentation with a right iliac fossa mass is common, occurring in 25% of patients in the NCI series, [41,209] and can be confused with an inflammatory appendiceal mass. Frequently at surgery there are multiple enlarged mesenteric lymph nodes that may or may not contain microscopically visible tumor, and multiple peritoneal plaques of tumor may be observed. Involvement of retroperitoneal structures, including kidneys and pancreas, is frequent, whereas liver and splenic involvement are seen somewhat less frequently. Ovarian involvement is common. Pleural effusions and ascites, which nearly always contain BL cells, and bone involvement (not infrequently multiple) are often present. Pharyngeal or nasopharyngeal sites of disease as well as paranasal sinus involvement are all occasionally seen. Thyroid and salivary gland involvement is uncommon. Unusual presentations of BL include isolated tonsillar (uni- or bilateral) involvement, sometimes only recognized after tonsillectomy for presumptive tonsillitis, and isolated cervical adenopathy. Testicular involvement at presentation occurs in a few percent of patients and skin and mediastinal involvement is very rare, but not unknown. Breast involvement, although rare, occurs nearly always in pubertal girls and pregnant or lactating women in both sporadic and endemic BL. [210,211,212 and 213] Jaw involvement in sporadic BL, in contrast to endemic BL, occurs in less than 10% of patients at presentation and is not age related. [214] Such patients frequently have involvement of other bony sites or of the bone marrow, suggesting that jaw involvement arises for quite different pathobiological reasons than it does in African patients. [215]

Bone marrow involvement is perhaps more common in sporadic BL than had previously been thought. At presentation, marrow involvement occurs in 20% of patients, [206,216] but there is evidence from *in vitro* culture and karyotyping of microscopically uninvolved bone marrow that occult involvement occurs in another approximately 20% of patients. [217] Some patients present with a clinical syndrome consistent with leukemia without any solid lymphomatous masses apart from lymphadenopathy and hepatosplenomegaly. These patients, in the past, constituted 2% to 5% of most large series of patients with ALL, and were referred to in the French-American-British classification as *L3 ALL*. These and all other patients with greater than 25% bone marrow involvement have been more generally referred to as having *acute B cell leukemia* in more recent years, and might better be referred to as *Burkitt's leukemia* to prevent the misconception that such patients should be treated with ALL therapy. If such cases are taken into consideration, involvement of the bone marrow in BL occurs in a rather high fraction of cases in the United States and Europe (in published series from other countries, however, bone marrow involvement is quite uncommon [60,62,219]). Furthermore, in BL in the United States,

involvement of the bone marrow is frequently observed at relapse such that the majority of patients who die from tumor progression have had bone marrow involvement at some time in the course of their illness. It should be pointed out, however, that not all acute leukemias that conform to the criteria of L3 morphology or express surface immunoglobulin have the phenotypic and genetic changes of BL. [220,221,222 and 223] Conversely, there are occasional reports of leukemias with an 8;14 translocation and precursor B-cell morphology. [224,225]

In contrast to endemic BL, CNS involvement and epidural tumor is quite uncommon at presentation in sporadic BL but is distinctly more common in the presence of bone marrow involvement.[216,226,227] Approximately two-thirds of patients with bone marrow disease may have simultaneous CNS involvement. Compression of the inferior alveolar nerve as it passes through the mandible, with resultant numbness of the lip and chin, occurs quite frequently in patients with widespread bone marrow disease, and this, as with compression of cranial nerves by orbital tumor, is not a sign of CNS involvement, although such patients are at high risk to develop CNS disease. Presentation with cranial nerve involvement is occasionally observed, along with cerebrospinal fluid (CSF) pleocytosis, and the latter may be the only indication of CNS (meningeal) disease. Involvement of cranial nerves without evidence of systemic disease is remarkably uncommon but has been described, [228,229] as has a syndrome of multiple peripheral nerve involvement. Intracranial tumor, when it does occur, is more likely to be extradural, although it can be within the brain parenchyma.[230]

### *Endemic Burkitt's Lymphoma*

In patients with BL in equatorial Africa, jaw involvement of multiple jaw quadrants in a large proportion, is the most frequent site of tumor, although it is very much age dependent, occurring particularly in young children ( Fig. 24-12). In an early series collected by Burkitt,[7,231] 70% of children younger than 5 years with BL had jaw involvement as compared to 25% of patients older than 14 years.[28] In very young children, orbital involvement is often present in patients who do not have jaw tumors, although at least some of these orbital tumors arise in the maxilla. Maxillary tumors are twice as frequent as mandibular tumors in endemic BL. In the endemic region of Africa itself, the percentage of patients with jaw tumors appears to be inversely proportional to the incidence of the disease. In addition, the median age of patients tends to be higher in lower incidence areas such as highlands or arid regions. [232] Abdominal involvement is also frequent in endemic BL, being present in just over half the patients and frequently invading the mesentery and omentum, but involvement of the right iliac fossa (appendiceal/cecal region) is uncommon. [28,232] Liver and spleen are not frequently involved. Thyroid and salivary gland involvement is not uncommon, and adrenal gland involvement is occasionally seen. [28,231,232] Isolated lymphadenopathy, tonsillar (or any form of pharyngeal tumor), and splenic involvement are all extremely rare in African BL. Involvement of the ovary is frequent, but testicular involvement is uncommon. Breast involvement, as in sporadic tumors, occurs almost exclusively in pubertal girls or lactating women. [234,235] Involvement of the skin, testis, and pericardium is occasionally observed, but mediastinal tumor is essentially unknown. [28,231,232]

**FIGURE 24-12.** An African child with Burkitt's lymphoma that involves the maxilla.

Bone marrow involvement occurs in only approximately 8% of patients with endemic BL at presentation, but there are no estimates of the proportion of patients who have occult bone marrow involvement, nor is the frequency of L3 or acute B-cell leukemia, presenting as ALL, known in Africa. [28,216] The rarity with which BL recurs in the bone marrow in African patients, even after multiple relapses, further confirms, however, its lack of predilection for the bone marrow. It is worth pointing out that in patients with jaw tumors the marrow of the jaw is clearly infiltrated at a microscopic level, whereas diffuse marrow involvement is seldom observed.

CNS disease, either CSF pleocytosis or cranial nerve palsies, is much more frequently observed in endemic BL than in the sporadic variety, occurring in approximately one-third of patients at presentation in one Ugandan series. [236] Any cranial nerve can be involved, but the ophthalmic nerves and the facial nerve are more often affected.[28,236] Very rarely, cranial nerve involvement and CSF pleocytosis have been the sole sites of disease [237] and peripheral neuropathy is occasionally seen.[238] Also rare, even in the presence of other cranial neuropathies, the optic nerve may be infiltrated giving rise to blindness. In Uganda, approximately 15% of patients present with isolated epidural lymphoma and paraplegia, requiring laminectomy for diagnosis. [28,233,236] Intracerebral disease has been described but is usually diagnosed only at recurrence.[239]

A comparison of sites of disease at presentation in endemic (Ugandan) and sporadic (United States) BL is shown in Table 24-4. In addition to the differences in jaw involvement, there are striking differences in the frequency of involvement of the bone marrow (higher in sporadic); CNS, including spinal epidural disease (higher in endemic); and other sites (those involved in mucosa-associated lymphoid tissue lymphomas, such as the thyroid and salivary glands).

| Site[c] | Uganda (percentage of 224 patients) | United States (percentage of 135 patients) |
|---|---|---|
| Jaw | 58 | 14 |
| Abdomen/pelvis | 58 | 60 |
| Cerebrospinal fluid/cranial nerves | 19 | 11 |
| Paraspinal sites | 17 | 2 |
| Orbit | 11 | 5 |
| Bones | 9 | 24 |
| Thyroid | 8 | 0 |
| Bone marrow | 7 | 21 |
| Salivary glands | 5 | 0 |
| Peripheral lymph nodes | 4 | 42 |
| Pleural effusion | 3 | 26 |
| Skin/soft tissues | 3 | 5 |
| Testis | 2 | 6 |
| Breast | 2 | 4 |
| Mediastinal nodes | 1 | 12 |
| Nasal sinuses | <1 | 3 |
| Pharynx | 0 | 10 |

[a]Uganda Cancer Institute series.
[b]National Cancer Institute, Bethesda, series.
[c]Detection of sites differed in the African series largely by clinical examination supplemented by chest x-ray films, intravenous pyelogram, bone marrow, and cerebrospinal fluid examination. In the U.S. series, computed tomography and nuclear medicine scans were performed, as well as cerebrospinal fluid and bone marrow examination.

**TABLE 24-4. RELATIVE FREQUENCY OF INVOLVEMENT OF DIFFERENT SITES AT PRESENTATION IN ENDEMIC BURKITT'S LYMPHOMA[a] VERSUS SPORADIC BURKITT'S LYMPHOMA[b]**

### *Other Populations*

Patients with BL in North Africa, the Middle East, and South America appear to have a spectrum of organ involvement that more closely approximates that of the sporadic disease rather than the endemic form—that is, jaw tumor is infrequent, and most patients present with abdominal tumor, [219,240,241,242,243,244,245,246,247 and 248] although higher percentages of patients with jaw tumors have been reported in some Asian countries, South Africa (in which both whites and nonwhites appear to have as high a frequency of jaw tumors as that in the endemic form of the disease), Turkey, Japan, and equatorial Brazil. [28,245,247,248 and 249] Paraplegia occurs occasionally, again suggesting that the “endemic form” of BL may occur at low incidence outside Africa. [250] Bone marrow involvement has been reported to occur with lower frequency in several series from outside the United States or Europe, as is the case in equatorial Africa. [60,62,251] These differences may be real or due to exclusion (or referral to a different specialist) of cases with overt leukemia from series of patients with BL, although the latter explanation is unlikely to account for the

finding in Africa, in which childhood leukemia is itself uncommon.

### Burkitt's-Like and Large B-Cell Lymphomas

In spite of the problem of reproducibly separating BL from BLL and BLL from LBCL,[252] there is no doubt that BLL and LBCL have a broader range of presentations than BL. Because BLL includes at least a proportion of cases that resemble, at a molecular genetic level, LBCL,[189] and this proportion increases with age, it is probably true that the older the patient, the more likely the presentation to resemble that of LBCL rather than BL. In addition, there is considerable overlap in the sites of disease at presentation even in classic BL and classic LBCL. Nonetheless, BLL and LBCL much more frequently involve lymph nodes than does BL, whether peripheral, intrathoracic, or intraabdominal, and these sites, as well as liver and spleen, appear to be more often involved in older than in younger individuals. BLL and LBCL also present quite frequently with extranodal disease in sites typical of BL, particularly in the abdomen (bowel, mesentery and retroperitoneum), and involvement of almost any of the sites listed above for BL has been observed. Some sites, such as the mediastinum, are considerably more frequently involved than in BL. The majority of such lymphomas falls into the special category of LBCLs of the mediastinum,[253,254] and[255] and these tumors, which may be associated with superior vena caval (SVC) obstruction and pleural and pericardial effusions, rarely involve lymph nodes. If spread beyond the mediastinum, they are more likely to involve kidneys, adrenals, liver, and ovaries, although bone marrow involvement is uncommon. In BLL and LBCL, bone marrow involvement is quite common, but CNS disease may be rather less commonly present than is the case for BL. In the immunosuppressed individual, isolated involvement of the brain by LBCL is frequent.

### Lymphoblastic Lymphomas

Patients with LLs of precursor T-cell origin most commonly present with intrathoracic tumor, particularly a mediastinal mass (50% to 70%) frequently associated with pleural effusions.[112] Symptoms may include pain, dysphagia, dyspnea, or swelling of the neck, face, and upper limbs from SVC obstruction. Inferior vena caval obstruction occasionally occurs due to compression of the vein as it traverses the diaphragm, but it is rarely symptomatic. In Europe and the United States, if patients with intrathoracic LL have lymphadenopathy (which occurs in 50% to 80% of patients), it is likely to be above the diaphragm, in the neck, the supraclavicular regions, or the axillae. Pericardial tumor or effusion can occur, sometimes with significant cardiac tamponade. Abdominal involvement is quite uncommon and almost never massive, being more likely to be manifested simply as hepatic or splenic enlargement, or a retroperitoneal mass detected with special imaging techniques. LL arising in the retroperitoneum is more likely to have immunophenotypic features consistent with NK cells.[256] Generalized peripheral lymphadenopathy is occasionally seen but should raise suspicions of bone marrow involvement. These and other “peripheral” sites of disease, including bone, testis, nasopharynx, and skin are not usually associated with a large mediastinal mass, suggesting biologic differences between clinical subtypes of LL.

LL of precursor B-cell phenotype nearly always presents with limited disease, with frequent involvement of bone, isolated lymph nodes, or soft tissues, including skin.[257,258,259,260,261] and[262] More extensive disease would almost certainly include diffuse bone marrow involvement—that is, would be diagnosed as ALL.

In LL it is difficult to give a figure for the frequency of bone marrow involvement because this depends entirely on the definition of the disease. In the United States and United Kingdom, T-cell ALL accounts for 12% to 15% of all cases of ALL, and this form of T-cell acute lymphoblastic neoplasia is more common than LL when the latter is diagnosed on the usual basis of having less than 25% of blasts in the bone marrow. In earlier series of patients with LL (i.e., treated inadequately by today's standards), bone marrow involvement occurred at some point in the course of the disease in more than 50% of cases,[20,263] but CNS involvement, including CSF pleocytosis, cranial nerve involvement, or the rare intracranial disease, is present in less than 10% of patients with LL at presentation.

### Anaplastic Large Cell Lymphoma

Unlike the other NHLs of childhood, ALCL can present with a slowly progressive, even waxing and waning course. This is often associated with systemic symptoms such as fever and weight loss. Among the most frequent sites of involvement (again, in contrast to other childhood NHLs, which are predominantly extranodal) are lymph nodes, both peripheral and intrathoracic or intraabdominal.[264,265] Skin involvement is much more frequent than in other childhood NHLs, particularly the skin of the lateral thorax. Involvement of lymph nodes and skin was a prominent feature in the first series of children recognized as having this disease.[205] Bone involvement is also common and often multiple, and some patients present with primary bone involvement.[266] Primary cutaneous ALCL is less common in children than in adults, but has been described.[267] Mediastinal involvement and hepatomegaly or splenomegaly are often present, and the tumor may also involve unusual sites for lymphoma, such as muscle, lung parenchyma, and other soft tissues. In contrast, the gastrointestinal tract is rarely involved and the CNS and bone marrow are both unusual sites of disease, although several cases of the small cell variant of ANLC in which there was a leukemic presentation have been recently described.[268]

## METHODS OF DIAGNOSIS

Histologic analysis remains the primary mode of definitive diagnosis for a child with a suggestive clinical syndrome or, less frequently, persistent lymph node enlargement, but it is important to supplement this information with phenotypic and, wherever possible, cytogenetic examination. Distinction between lymphomas and other “small round blue cell tumors,” which include round cell sarcomas (e.g., Ewing's sarcoma and some rhabdomyosarcomas) and neuroblastoma, is usually not difficult on histologic grounds alone, but where there are difficulties, immunophenotyping, if necessary coupled to molecular studies, is nearly always sufficient to resolve diagnostic problems. The presence of the leucocyte common antigen CD45,[269] which is not present on nonhematologic neoplasms provides sufficient confirmation of a lymphoid cell population. Relatively specific markers now exist for many of the round cell tumors, but care is required in the overall interpretation. For example, the MIC-2 protein that is expressed by Ewing's sarcoma and primitive neuroectodermal tumors may also be expressed on NHLs, particularly LL, although rarely on BL.[270,271] Detection by PCR of the fusion genes generated by chromosomal translocations in Ewing's sarcoma, primitive neuroectodermal tumor, and alveolar rhabdomyosarcoma, has essentially eliminated the diagnostic problem of the small blue round cell tumor. The examination of CSF by PCR may also reveal unsuspected CNS involvement, and the detection of EBV DNA in CSF has been used to confirm the presence of CNS lymphoma in acquired immunodeficiency syndrome patients.[272,273,274] and[275] Tests for the presence of EBV in tumor cells by *in situ* hybridization, generally by detecting the presence of the highly expressed small EBV RNAs, can also be helpful in the diagnosis of other lymphomas, particularly posttransplant and HIV-associated lymphomas.

The distinction between lymphoma and a non-neoplastic lymphoproliferative process is rarely difficult, particularly when immunophenotyping results are available. Occasional diagnostic problems may occur with ACLC, particularly in those with a null phenotype. The presence of CD30 is insufficient evidence of this disease—mediastinal LBCL, for example, quite frequently expresses CD30, albeit usually at a lower level than in ALCL.[276] Although expression of EMA may be helpful, the demonstration of a 2;5 translocation or, more rapidly, expression of the protein product of this translocation, p80, by immunohistochemistry, is diagnostic, although the latter is not present in 100% of ALCLs. Lymphomatoid papulosis, a generally benign disease confined to the skin, does have somewhat different histologic characteristics, but it is cytologically indistinguishable from ALCL and is a trap for the unwary. It may have a waxing and waning course, uninfluenced by chemotherapy, and only occasionally develops into ALCL. As with ALCL confined to the skin, however, lymphomatoid papulosis does not generally contain 2;5 translocations, although some authors have found such translocations in approximately 10% of both lymphomatoid papulosis and the form of ALCL confined to skin.[277,278] and[279]

One area in which there is often difficulty in deciding whether the condition is a malignant lymphoma or benign lymphoproliferative condition is in the child with an underlying immunologic disorder that may include long-standing lymphadenopathy. Demonstration of clonality (by detecting the expression of a single light chain by immunophenotyping, or the presence of immunoglobulin or T-cell receptor gene rearrangements at a molecular level) is helpful, and detection of a specific translocation, diagnostic in making this distinction. It should be recognized that even so-called benign lymphoproliferative syndromes can be lethal, however, and in some circumstances a clinical trial of therapy (e.g., interferon-a or a B-cell monoclonal antibody) may be appropriate while assessing the clinical course. Rapidly progressive disease may be an indication for therapy with cytotoxic drugs.

At the time of biopsy of a suspected pediatric neoplasm, tissue should be provided, unfixed, to the pathologist so that some can be frozen, some used for immunologic and molecular studies, and some processed for karyotyping. The material used for these special studies must contain minimal amounts of normal tissue unless molecular or combined morphologic and phenotyping studies are to be performed, in which circumstances clonal populations can be detected even if representing only a small percentage of the total number of cells. Southern blotting, for example, can distinguish a clonal population representing only 5% of the cells present, whereas PCR can detect a specific translocation even if only a single translocation is present in the DNA sample used for the test. As microarray techniques develop, it is probable that new genes of diagnostic significance, whether single or multiple (“signature sets”), will be identified and used in diagnosis either via small arrays or in the context of standard immunohistochemistry or PCR.

Serum studies cannot provide diagnostic information for lymphomas, but they may provide evidence of a nonlymphoid origin when, for example, there are high levels

of catecholamines or their metabolites, or other tumor markers such as alpha-fetoprotein or carcinoembryonic antigen. Lactate dehydrogenase elevations are nonspecific, and this, or the detection of high serum levels of soluble interleukin-2 receptor and other molecules associated with lymphoid cells, such as $b_2$-microglobulin, are generally more useful in the provision of prognostic rather than diagnostic information because of their nonspecificity. [280,281]

## MANAGEMENT

The management of children with NHL includes initial assessment, to determine whether emergency measures are necessary; the determination of the extent and sites of disease, to assess prognosis and to determine optimal therapy; and the initiation, at the earliest time after diagnosis, of specific therapy.

### Staging

Staging systems for childhood NHL were designed to identify patients who fall into different prognostic risk categories. The major determinants of prognosis in childhood NHL are the treatment given, the biology of the tumor, and the extent or volume of tumor. Tumor biology is reflected in histology so that tumors of different histologic types often differ in their responses to various treatment approaches, even when tumor volume is taken into consideration. Of course, even tumors of the same histology and tumor burden may have very different responses to therapy, because one may prove resistant to treatment. At the present time, there is no way other than initial response to therapy [poor response to pre-therapy is used, for example, by the French Society of Pediatric Oncology (SFOP) to identify high risk patients who then receive more intensive therapy] to definitively identify patients destined to relapse or die from progressive disease when further treated with the same therapy, although the *risk* of relapse and survival is correlated with the tumor burden. Perhaps in the future, the identification of sets of signature genes (e.g., by microarray or proteomics) that, when expressed (or not expressed), are associated with a poor prognosis (in the context of a specific treatment protocol) may markedly improve the ability to predict differences in outcome stemming from tumor biology. Other than this, tumor volume, as measured by staging and biochemical measures such as lactate dehydrogenase (LDH) (or in the African patient, anti-EBV antibody titers) remains the most important determinant of response to a given therapy.[282] Comparison of tumor burden by staging and LDH is, therefore, essential if the results of one therapy are to be compared with another (in the same histologic category of tumor), particularly when the series is not large.

The most widely used staging classification today is that introduced by the St. Jude Children's Research Hospital, a scheme based on the Ann Arbor staging system for Hodgkin's disease, modified for NHL. The St. Jude system is applicable to all histologic types of childhood lymphoma and separates patients with limited-stage disease (i.e., one or two masses on one side of the diaphragm) from those with extensive intrathoracic or intraabdominal disease, although the meaning of *extensive* is not defined. Perhaps the biggest problem with this staging system is that stage III encompasses patients with a wide range of tumor burdens. Indeed, various attempts have been made to distinguish subtypes of stage III in B-cell lymphomas. Recently, the Berlin-Frankfurt-Münster (BFM) group showed that a serum LDH level above 500 U per L is an important negative prognostic factor, and that by treating such patients with ten times the dose of MTX, markedly improved results could be achieved[283] Patients with bone marrow infiltration with less than 25% tumor cells seen on aspirate and patients with involvement of the CNS are separated into the worst prognostic group, stage IV (Table 24-5), although the very intensive therapy regimens used today have resulted in markedly improved results for such patients, often not significantly different from those of stage III.

| Stage | Description |
|---|---|
| I | A single tumor (extranodal) or single anatomic area (nodal), excluding mediastinum or abdomen |
| II | A single tumor (extranodal) with regional node involvement<br>On same side of diaphragm:<br>(a) Two or more nodal areas<br>(b) Two single (extranodal) tumors with or without regional node involvement<br>A primary gastrointestinal tract tumor (usually ileocecal) with or without associated mesenteric node involvement, grossly completely resected |
| III | On both sides of the diaphragm:<br>(a) Two single tumors (extranodal)<br>(b) Two or more nodal areas<br>All primary intrathoracic tumors (mediastinal, pleural, thymic)<br>All extensive primary intraabdominal disease; unresectable<br>All primary paraspinal or epidural tumors regardless of other sites |
| IV | Any of the above with initial central nervous system or bone marrow involvement (≥25%) |

**TABLE 24-5. THE ST. JUDE STAGING SYSTEMS FOR CHILDHOOD NON-HODGKIN'S LYMPHOMA**

Because of the rapidity of tumor growth in children and adolescents with NHL, it is important to expedite staging procedures and other investigations indicated by the history and examination of the patient, because delay increases the risks of a complication due to compression of adjacent anatomical structures. It is inappropriate to delay therapy to identify sites of additional disease that would not influence the approach to therapy. Staging laparotomy is not advocated for patients with NHL, although a fraction of patients will have had laparotomy for diagnostic purposes. The first component of staging is the history and clinical examination, supplemented when appropriate by special procedures such as endoscopic examinations—for example, for pharyngeal tumor or upper gastrointestinal bleeding. Additional components include imaging, examination of the bone marrow and CSF, and biochemical and hematological tests.

Modern imaging methods, including ultrasonography and computed tomography (CT) provide adequate means of evaluating disease sites. [284,285] Ultrasonography has advantages over CT scanning when retroperitoneal fat is minimal, as in small children, and in most cases may provide sufficient information for determination of the appropriate treatment group. It may also be useful in defining or detecting testicular masses, but the rarity of testicular involvement at presentation argues against its routine use for this purpose. CT scans may be particularly useful in determining the presence of residual disease after surgery if this is a critical factor in assignment to treatment groups (as it usually is). Gallium scanning provides a useful whole-body screen, particularly for BL and BLL, which avidly take up the isotope. Scanning techniques have been improved considerably in recent years, and both the administration of higher doses of gallium and the use of single-photon emission CT scanning has considerably increased the value of this procedure. [286,287 and 288] Moreover, gallium uptake provides additional information useful for determining response to treatment, because it is not normally taken up by nonviable tumor such that the clinical significance of residual masses identified on radiological imaging can be better assessed.[287,288,289 and 290] A bone scan is the most sensitive means of detecting bony involvement, but it often adds little to the information provided by a gallium scan. Radionuclide liver and spleen scans also add little to CT and ultrasound images. Magnetic resonance imaging has some advantages with respect to imaging certain sites or organs and is particularly useful for evaluating the CNS, but because CNS involvement is rare at presentation, it is generally not included as part of a standard staging system (although it should be performed in the presence of symptoms or signs suggesting CNS disease).

Bone marrow and CSF examination are an essential part of staging, although when prophylactic intrathecal drug administration is simultaneously initiated with systemic therapy, the initial CSF examination can be carried out at that time. Bilateral bone marrow aspirates and biopsies are superior to a single aspirate in detecting bone marrow disease because of frequent discrepancies between aspirates and biopsies and between left and right samples, [291] although because with most modern treatment protocols the presence of limited bone marrow disease has no measurable impact on outcome in patients who would otherwise be stage III, its value in this context is questionable, and many would not advocate multiple biopsies on a routine basis.

Quantitative biochemical (e.g., serum LDH) or immunochemical measurements, as implied, provide a simpler and more objective measurement of tumor volume, although occasionally, patients with extensive disease do not have an elevated serum LDH level. Such measurements should be included in the evaluation of the patient at initial presentation, and serum LDH has become an accepted component of risk assessment for treatment assignment in BFM protocols.

Abnormal liver function tests suggest hepatic involvement, and renal function tests (including assessment of urine output) and measurement of serum uric acid level are essential for determining the presence of uric acid nephropathy as well as the likelihood of the development of a tumor lysis syndrome (see the section on hyperuricemia and the acute tumor lysis syndrome). Renal function tests are not usually helpful in assessing the presence of renal involvement because of the high likelihood of uric acid nephropathy in patients with very extensive disease even in the absence of renal involvement, but they are essential to patient management. An acceptable list of investigations for staging purposes is shown in Table 24-6.

| | |
|---|---|
| ***Important investigations*** | |
| Physical examination | |
| Complete blood cell count | |
| Chemistries | |
| Serum electrolytes | |
| Liver and renal serum chemistries | |
| Serum lactate dehydrogenase | |
| Serum uric acid | |
| Imaging studies | |
| Chest x-ray | |
| Chest CT scan (if chest x-ray film abnormal or suspiciously abnormal) | |
| Thoracic ultrasound (e.g., for following thoracic tumor) | |
| Abdominal ultrasound examination (include liver/spleen, kidneys, abdomen, pelvis)[a] | |
| Gallium-67 scan | |
| Abdominal CT scan (can be waived if ultrasound and gallium scan performed) | |
| Bone marrow examination (bilateral biopsy and aspirates) | |
| Cerebrospinal fluid examination (cytology) | |
| ***Useful or sometimes indicated investigations*** | |
| Serum lactate (especially in the presence of a large tumor) | |
| Bone scan (for more precise documentation of bony lesions) | |
| Magnetic resonance imaging scan for suspected central nervous system disease or if indicated | |
| Endoscopy (e.g., for gastrointestinal bleeding) | |

CT, computed tomography.
[a]MRI be the better study in children with little intraabdominal fat and can be useful in differentiating between bowel and tumor.

**TABLE 24-6. INVESTIGATIONS REQUIRED FOR ACCURATE STAGING OF CHILDHOOD LYMPHOMAS**

## Risk Assignment

Modern protocols divide patients into at least two, and more often three or four groups for the purposes of assigning them to an appropriately intensive therapy arm (Table 24-7, Table 24-8, and Table 24-9). Patients with minimal disease require less intensive therapy than do patients with extensive disease, and because of the risks of therapy—both acute and chronic toxicities, as well as the inconvenience and discomfort related to more intensive therapy—it is important not to administer more intensive therapy to patients than they need. At the present time, however, risk group assignment, although following similar principles, differs in different protocols and in different cooperative groups. In general, patients with B-cell tumors in which all disease has been surgically removed, particularly if of small volume (nearly always the case) before surgery, are considered to be in the lowest risk group. Typically, such patients receive only two or three therapy courses. Patients with extensive disease, as measured by stage and serum LDH level, as well as the presence of CNS disease are generally considered to be in the highest risk group and are treated with the most intensive therapy, usually involving more than six drugs and four or more cycles of therapy. Patients with bone marrow involvement are also at high risk but are sometimes (e.g., in SFOP studies) subdivided according to the extent of bone marrow involvement; in the latter case, only patients with more than 70% tumor cells in the bone marrow were, until recently, classified as being in the highest risk category. Patients not in the lowest or highest risk groups are assigned to intermediate risk groups.

| Therapy group | Definition |
|---|---|
| A | Complete surgical resection stage I or abdominal stage II |
| B | All patients not eligible for group A or C |
| C | Any central nervous system[b] involvement or bone marrow involvement ≥25% blasts |

[a]Identical to U.K. and U.S. Children's Cancer Study Group protocols CCG5961 and UKCCSG NHL 9600.
[b]Central nervous system involvement is defined as any blasts in cerebrospinal fluid, cranial nerve palsies not explained by extracranial tumor, clinical spinal cord compression, isolated intracerebral mass, parameningeal extension, cranial or spinal involvement.

**TABLE 24-7. DEFINITIONS OF THERAPY GROUPS IN FRENCH-AMERICAN-BRITISH PROTOCOL LYMPHOME MALIGNE B 96 FOR B-CELL LYMPHOMAS[a]**

| | |
|---|---|
| **Lymphoblastic lymphoma** | |
| Low risk | Stages I and II |
| Intermediate risk | Stages III and IV |
| High risk | Residual tumor of >30% after phase I of protocol I (day 33)<br>>5% of blast cells in bone marrow on day 33 of protocol I<br>Persistent blast cells in cerebrospinal fluid on day 33<br>Tumor progression during protocol I |
| **Burkitt's and large B-cell lymphoma** | |
| Risk group 1 | Complete surgical resection |
| Risk group 2 | Incomplete surgical resection<br>Stages I and II<br>Stage III and LDH <500 U/L |
| Risk group 3 | Incomplete surgical resection<br>Stage III and LDH 500–999 U/L<br>Stage IV or B-cell leukemia and LDH <1000 U/L; CNS negative |
| Risk group 4 | Incomplete surgical resection<br>Stage III and LDH >999 U/L<br>Stage IV or B-cell leukemia and LDH >999 U/L; positive or negative CNS |

BFM, Berlin-Frankfurt-Münster; CNS, central nervous system; LDH, lactate dehydrogenase.
[a]Non-Hodgkin's Lymphoma–Berlin–Frankfurt–Münster 95.

**TABLE 24-8. DEFINITIONS OF THERAPY ARMS IN BFM PROTOCOL[a]**

| Therapy group | Definition |
|---|---|
| Low-risk patients | Stage I or II and LDH <150% of normal |
| High-risk patients | All other patients |

LDH, lactate dehydrogenase.

**TABLE 24-9. DEFINITIONS OF THERAPY GROUPS IN U.S. NATIONAL CANCER INSTITUTE PROTOCOL 89-C-41 FOR B-CELL LYMPHOMAS**

In patients with ALCL or LL, risk group assignment is less well studied, but patients with ALCL are usually divided in a way similar to those with B-cell lymphomas. Patients with LL rarely have limited disease, because the majority is of T-cell origin, and have "extensive" mediastinal masses. Those with limited disease, for example, localized bone or skin involvement, generally prove to have precursor B-cell LL, and many would advocate treatment with a protocol designed for low-risk ALL, with relatively prolonged therapy. In the Pediatric Oncology Group, patients with limited LL have been shown to require more prolonged therapy than do patients with limited B-cell lymphomas,[38,39] but they are sufficiently few in number that good data regarding the optimal duration of therapy are not presently available.

## Emergency Management

The need for emergency management arises quite frequently in pediatric NHL, because these tumors have very high growth fractions and doubling times. African BL has been estimated to have a potential doubling time as short as 24 hours, and cutaneous tumors have been observed to double in volume in 3 days. [292] Life-threatening complications may develop as a consequence of the physical encroachment of tumor masses on vital structures or because of high cell turnover in a large tumor, with resultant biochemical disturbances.[208] Airway obstruction may result from pharyngeal or intrathoracic masses; SVC obstruction and esophageal

compression from mediastinal masses; respiratory compromise from massive serous effusions related to involvement of the pleurae or peritoneum; cardiac tamponade or arrhythmias from pericardial tumor, pericardial effusion, or even myocardial involvement; paraplegia from epidural tumor; raised intracranial pressure and neurologic deficits from intracranial lymphoma; obstructive jaundice and pancreatitis from compression of the bile or pancreatic ducts; gastrointestinal bleeding, obstruction of the bowel, and rarely, perforation, from intestinal involvement; renal failure from renal outflow tract obstruction or, rarely, massive renal involvement; and inferior vena caval obstruction and lymphedema from retroperitoneal tumor. Severe pain may result simply from rapid expansion of tumor growing within a confined space, including the abdomen (particularly when there is massive ascites), breasts, brain, or a limb compartment (uncommon). Although venous obstruction of the great veins is relatively common, obstruction of peripheral veins or arteries is rare but not unknown (e.g., from tumor in the popliteal fossa), and occasionally peripheral neuropathy can result from compression of nerves by a mass or from direct infiltration of nerves, particularly nerve roots in the brachial and sacral plexuses. This may be associated with pain as well as motor and sensory impairment.

Involvement of the bone marrow can give rise to anemia, neutropenia with a consequent risk of infection, or thrombocytopenia and a risk of bleeding. Electrolyte or biochemical abnormalities of serious consequence (e.g., uricocemia renal failure, lactic acidosis) may be associated with large tumor volumes. Rarely, because of the production by tumor cells of molecules with hormone-like activities, hypoglycemia or hypocalcemia may lead to a medical emergency. [208] Fever, weight loss, and night sweats are occasionally caused by the tumor itself, rather than infection, particularly in the case of ALCL.

Because of the differences in the patterns of presentation of different types of lymphoma, the pattern of complications is, to a considerable extent, contingent on histology. Thus, compression of intrathoracic structures is much more likely with LL than with BL or BLL, whereas the reverse applies to intraabdominal complications. Large cell lymphomas, however, may occur in either the chest or the abdomen, and ALCL is more likely than are the other major histologic types of NHL to involve unusual sites, including skin. In addition, because the doubling time of BL tends, on average, to be shorter, and possibly because of biochemical differences, BLs are much more likely than are other tumors to present with electrolyte or biochemical abnormalities than other NHLs. [293]

Although immediate intervention may be required in some circumstances (e.g., tracheotomy, establishment of a diuresis, or hemodialysis) it must be emphasized that all of these complications can ultimately be dealt with only by reduction of tumor bulk, and delay will compound the problem, so the initiation of specific therapy as rapidly as possible must always be a primary goal. Clearly, in high-risk patients, even in the absence of overt complications, induction therapy is best administered in an intensive care unit. The management of the some of the more frequently encountered complications at presentation is discussed further in the sections that follow.

***Management of the Complications of Mediastinal Lymphoma***

The most serious complications of mediastinal masses are airway obstruction and cardiac tamponade. SVC obstruction is relatively frequent but is not, per se, a life-threatening complication, although its presence should lead to a careful assessment of airway competence. Dysphagia from esophageal compression may occur and can lead to discovery of a mediastinal mass but rarely becomes sufficiently severe as to require intervention, and is usually a less prominent symptom than is airway or SVC obstruction at the time of presentation, presumably because of anatomical considerations. Although clinical symptoms and signs are present with severe airway obstruction, narrowing of the trachea or major bronchi can occur without significant compromise of breathing while at rest. The most accurate method of assessing the anatomical degree of compression of the major airways is by CT scan. Severe airway obstruction is usually best managed by the institution of specific therapy, although in urgent situations before the establishment of a diagnosis, the institution of corticosteroid or radiation therapy may be indicated. Every effort should be made to establish a diagnosis before the institution of therapy, but general anesthesia and even heavy sedation should be avoided in patients with large mediastinal masses or airway obstruction (sudden death associated with anesthesia has been reported), and in this circumstance, bone marrow examination, biopsy of a peripheral lymph node, or examination of pleural fluid for tumor cells using only local anesthesia may permit a diagnosis to be established. SVC obstruction from a wide range of tumors has been traditionally treated with radiation therapy, but in highly chemotherapy-responsive tumors such as LL, irradiation increases toxicity without therapeutic gain. In the event that no symptomatic improvement (or worsening) is observed within a few days of the initiation of specific chemotherapy in patients with large mediastinal masses and serious respiratory compromise, mediastinal irradiation can be considered, but this circumstance is rare indeed, and augers poorly for the long-term outcome. If radiation therapy must be used, relatively low-dose therapy (e.g., to a total of 1,200 cGy) is preferable, because radiation does not improve the long-term outcome but may decrease marrow tolerance to chemotherapy and will increase the risk of pulmonary and cardiac toxicity, especially in the presence of anthracyclines.

Cardiac tamponade from pericardial tumor or pericardial effusion is best managed by the rapid institution of specific therapy coupled to pericardial paracentesis when necessary. The insertion of a catheter suitable for continuous drainage into the pericardial sac may be appropriate if the reaccumulation of fluid is rapid, but other measures, such as the construction of a pleuropericardial window or pericardiodesis with sclerosing agents, is not recommended at the time of presentation or remission induction, because the problem will normally be resolved as soon as there is sufficient tumor regression. Similar considerations apply to the management of massive pleural effusions. Rarely, antiarrhythmic drugs may be required because of myocardial infiltration or compression.

***Management of the Complications of Intraabdominal Lymphoma***

Massive intraabdominal lymphoma is nearly always of B-cell type in children and is most likely to be diagnosed as BL or BLL. Obstruction of the bowel, ureters, inferior vena cava or other retroperitoneal veins and lymphatics, and intraluminal bleeding or perforation of the bowel from tumor necrosis are the most frequently encountered complications. Intestinal obstruction is most commonly due to intussusception resulting from the intraluminal projection of a small tumor mass, and some patients present with symptoms of acute appendicitis. An acute abdomen is a relatively common presentation of NHL in children and will dictate laparotomy, which both establishes the diagnosis and, in the majority of patients, also leads to complete resection of the tumor. Such patients, particularly those who undergo emergency surgery, enjoy an excellent prognosis because they usually have very little tumor. In patients with gastrointestinal bleeding, surgical intervention should be strongly considered before specific treatment because tumor necrosis after chemotherapy is likely to considerably worsen the bleeding. However, surgical resection to *prevent* gastrointestinal bleeding or perforation in patients with extensive bowel disease is not recommended because both complications are rare, and perforation after chemotherapy may be *more* likely in patients who have been operated on or are malnourished. [294]

Ureteric obstruction (rarely, urethral obstruction) which is, again, a problem that is appropriately managed by the institution of specific therapy, has additional implications because if associated with a large tumor burden, it is likely to be accompanied by hyperuricemia and occurrence of an acute tumor lysis syndrome (see the section on hyperuricemia and the acute tumor lysis syndrome) after the initiation of therapy. The major treatment strategy for dealing with hyperuricemia is the establishment of a substantial diuresis—something that may be impossible in the presence of ureteric obstruction, particularly if bilateral—accompanied by the administration of allopurinol to reduce serum uric acid or, if available, urate oxicase. [295,296] The latter enzyme has been available for the treatment of hyperuricemia for many years in France[297] and is probably the major reason that serious electrolyte imbalances, potentially resulting in death, have not been a significant problem in the SFOP series (see the section on hyperuricemia and the acute tumor lysis syndrome). When acute renal failure cannot be alleviated by the above measures, and particularly when severe obstruction is a major component of the problem, it is appropriate to initiate hemodialysis (or, if feasible, peritoneal dialysis) until uric acid, electrolytes, urea, and creatinine are approximately normal, followed by immediate initiation of specific chemotherapy. Continue dialysis as necessary according to serum chemistries. Although ureteric stents or nephrostomy have been successful in bypassing ureteric obstruction, such measures are potentially hazardous, because the risk of perforating a ureter during placement of a stent or leakage of urine from a nephrostomy (particularly if tumor is in close proximity) are significant. In countries with limited resources and no access to hemodialysis, however, the use of urinary bypass may permit the establishment of a diuresis and initiation of chemotherapy.

Venous obstruction within the abdomen is a potential problem from two perspectives. First, intraluminal thrombosis may be present, necessitating consideration of anticoagulation or physical containment of thrombus to prevent pulmonary embolus. Anticoagulation can be hazardous because of the accompanying risk of gastrointestinal bleeding from involvement of bowel or the presence of thrombocytopenia from bone marrow involvement or chemotherapy. Further, oral anticoagulation with coumadin is difficult to control when potentially hepatotoxic chemotherapeutic agents, for example, MTX, are to be administered, such that heparin is preferable (where available, low-dose heparin may be an appropriate choice). In patients with intraabdominal lymphoma and venous thrombosis, pulmonary embolus (which is certainly a significant risk) may be prevented by the emergency placement of an intraluminal filter below the renal veins in the inferior vena cava.

The second potential consequence of intraabdominal venous or lymphatic obstruction is the inability to establish an adequate diuresis to deal with associated hyperuricemia and to avoid the development of a tumor lysis syndrome after the initiation of chemotherapy because of the tendency of administered fluid to accumulate in the lower limbs. Careful management of fluid balance and appropriate use of diuretics will usually permit the establishment of a diuresis, but recourse to hemodialysis may, on rare occasions, be necessary.

***Hyperuricemia and the Acute Tumor Lysis Syndrome***

The extremely high growth fraction and cell turnover rate of the childhood lymphomas, which are higher in B-cell lymphomas than in T-cell neoplasms, [298] although possibly accounting for their high sensitivity to chemotherapy, leads to the potential for the development of renal complications resulting from the increased solute burden on the kidneys. Both uric acid nephropathy occurring before the commencement of chemotherapy, and the development, immediately after chemotherapy, of an acute tumor lysis syndrome are correlated with the tumor burden and do not occur in patients with completely resected or minimal disease. [293,299,300] Acute tumor lysis syndrome refers to the occurrence of acute oliguric renal failure caused by the high urinary concentrations of phosphates and oxypurines and by their precipitation in the renal tubules when their solubility products are exceeded ( Table 24-10).

| | Acute uric acid nephropathy | Phosphate nephropathy | Xanthine nephropathy |
|---|---|---|---|
| Potassium | ↔ | + | ± |
| Urate | ↔ | + | ± |
| Phosphate | + | ↔ | ± |
| Predisposing factors | Aciduria | Alkaluria | Allopurinol |
| Time of onset | Before and after starting therapy | Within 48 h after starting therapy | After starting allopurinol |
| Prevention | Hydration, alkalinization, allopurinol or urate oxidase | Hydration | Hydration |

+, increase in plasma level; ↔, marked increase; ±, slight or no increase.
Adapted from Veenstra J, Krediet RT, Somers R, et al. Tumor lysis syndrome and acute renal failure in Burkitt's lymphoma. Description of 2 cases and review of the literature on prevention and management. Netherlands J Med 1994;45:211.

**TABLE 24-10. CLINICAL CHARACTERISTICS OF RENAL FAILURE ASSOCIATED WITH TUMOR LYSIS SYNDROME**

Although it is important to initiate specific therapy as soon as possible, the administration of chemotherapy in the presence of a markedly elevated uric acid and impaired urinary output would be highly likely to result in the death of the patient, probably from profound hyperkalemia—it was this complication that originally led to the recognition of the syndrome.[301] Therefore, the biochemical abnormalities must be corrected before the initiation of specific therapy. This period of biochemical correction by means of intensive diuresis (up to 250 mL per $m^2$ per hour in patients at highest risk) accompanied by allopurinol or urate oxidase should be completed as expeditiously as possible, preferably within 24 to 48 hours, because continued tumor growth is likely to compound the problem. The avoidance of potentially fatal hyperkalemia, hyperphosphaturia and renal failure—which will compound both problems—is best accomplished by ensuring a high urine volume before the initiation of chemotherapy. In French and German pediatric cooperative group protocols (both of which are now used in many other countries) a prephase of low-intensity chemotherapy (cyclophosphamide with vincristine and corticosteroid) is given during the first week of therapy based on the hypothesis that it will reduce the rate of response and thereby lessen the risk of acute tumor lysis. In some patients, for example, those recovering from major surgery, such an approach may have additional advantages—that is, allow improved wound healing. Response to this phase of therapy also appears to be predictive of the ultimate outcome of therapy and has been used by the SFOP as a means of identifying high-risk patients (see below).[302] Tumor lysis may occur, however, despite the administration of such a prephase[293] and the low incidence of metabolic complications in the SFOP protocol appears to be largely due to the use of urate oxidase in French studies. [302] For these reasons, the use of a prephase should not be interpreted as lessening the need for hyperhydration before and during initial chemotherapy in patients with a moderate to large tumor burden. Because of the relatively poor solubility of phosphates in alkaline urine, it is preferable to maintain the urine pH at approximately 7 and not to administer bicarbonate during chemotherapy. At pH 7 uric acid is 10 to 12 times more soluble (solubility is 150 mg per L at pH 5) and xanthine more than twice as soluble (solubility at pH 5 is 50 mg per L) than at pH 5. The solubility of hypoxanthine differs little at either pH (140 to 150 mg per L). The administration of high-dose allopurinol (e.g., 10 mg per kg) ensures that a significant proportion of purine metabolites is excreted as xanthine and hypoxanthine. It is inadvisable to completely prevent uric acid production because it is more than ten times as soluble in urine than is xanthine and slightly more soluble than is hypoxanthine. The objective of allopurinol therapy, therefore, is to increase the total amount of oxypurine that can be excreted in a given volume of urine rather than to prevent uric acid formation. Urate oxidase can very rapidly decrease uric acid levels by conversion to allantoin, more than ten times as soluble, and simplify the management of patients with a high tumor burden. This enzyme is gradually being introduced into standard practice in other countries, [295,296] and the development of recombinant versions should make it more widely accessible.[297]

Because of the risk of sudden death from hyperkalemia, which may occur within hours of the initiation of therapy, [301] it is important to avoid potassium supplements shortly before and during the first few days of therapy except in exceptional circumstances. Ideally the patient should be mildly hypokalemic before the commencement of chemotherapy. Hyperkalemia, similar to the other complications of acute tumor lysis, is extremely unlikely to occur in the presence of a high urine output. Hypocalcemia, a consequence of hyperphosphatemia, should not be treated unless symptomatic, and intravenous calcium chloride should be given with great caution, if at all, because of the risk of extraosseous calcification in the presence of a high serum phosphate level. Systemic alkalinity increases the possibility of symptomatic hypocalcemia, including tetany, and this is another reason that alkalinization of the urine is recommended only before chemotherapy, not during therapy. Rarely, hemodialysis may be required for symptomatic hypocalcemia. Because of the quantity and type of monitoring required, it is preferable to manage patients at high risk for acute tumor lysis syndrome in a critical care unit.

### *Management of Neurologic Emergencies*

The primary neurologic emergencies encountered in children with NHL include paraplegia, cranial nerve palsies, meningeal disease, and intracerebral tumor. Although radiation would at one time have been considered appropriate therapy for each of these problems, this is no longer necessarily the case. Given the generally excellent responses to chemotherapy in all childhood lymphomas, there is no reason to presume that tumor will respond more rapidly to radiation. In fact the reverse is usually the case, although radiation response is histology dependent. Reversal of neurologic complications, including dense paraplegia (but only when of brief duration) as well as cranial nerve palsies, has been observed frequently in African BL treated with chemotherapy alone, [28] but radiation response, at least to standard fractions, is generally poor in BL.[36] Extradural disease, the usual cause of paraplegia, and cranial neuropathy, is amenable to standard systemic therapy, and emergency radiotherapy for these forms of CNS disease is not necessary. Failure of paraplegia to resolve may be due to compression of the arterial blood supply to the cord. In BL, there is also no evidence that irradiation is of value in the presence of CSF malignant pleocytosis, which in emergency situations can be effectively managed by intrathecal therapy,[227] although magnetic resonance imaging should be performed to exclude raised intracranial pressure before performing a lumbar puncture in suspected cases. Radiation, although it may have little effect on response rates, is likely to increase the risk of late complications. [303,304 and 305] This, coupled to the success of high-dose systemic cytarabine (Ara-C) and MTX in the treatment of patients with the most advanced disease has led to a significant trend away from the use of radiation for patients with BL and CNS disease at presentation. The role of radiation in the treatment of CNS involvement in other histologies is less clear, and unfortunately, there are too few patients in this situation for randomized studies to be conducted. [306] As with BL, the pros and cons include the curative potential of purely chemotherapeutic approaches weighed against the potential for increased acute and late toxicity of cranial irradiation in association with chemotherapy, particularly when high-dose systemic and intrathecal MTX or Ara-C are used. [305] It is of interest that even in the treatment of primary CNS lymphoma in adults, radiation gives very poor results when used alone, significantly worse than those achieved with high-dose MTX, providing further support for the omission of radiation therapy even for the emergency treatment of patients with CNS lymphoma.[307]

### Relative Importance of the Primary Treatment Modalities

The primary therapeutic modality for the NHLs of childhood is chemotherapy, regardless of stage or sites of disease. Theoretically justified on the grounds that the NHLs are disseminated diseases, this statement is also firmly rooted in empirical clinical experience. The average survival in a combined analysis of eight published series of children with localized NHL—a total of 370 patients—treated with radiation, surgery, and single-agent therapy was 18%. [308] Yet patients with a similar tumor burden treated with combination drug therapy with or without radiation have enjoyed a cure rate usually in excess of 90% for many years. [309,310,311 and 312] These data, coupled to data from Africa, in which poor response to radiation was documented (although there was evidence in these studies that hyperfractionation would be beneficial[36,313]), were sufficient to convince most that radiation probably has no role in the modern therapy of early stage disease, but confirmation has come from a randomized trial conducted in the United States in which radiotherapy was shown not only to have no advantage but also to be associated with a significant toxic cost when combined with a drug combination that cured approximately 90% of patients.[38,39] The lack of benefit of radiation in patients with localized disease would suggest strongly that patients with extensive disease would not benefit from radiation to sites of bulky disease either, and indeed, modern multidrug therapy protocols that do not include radiation clearly produce markedly better results in all histologies [302,314,315] than those of earlier protocols in which local irradiation was used in addition to combination chemotherapy.[305] Even patients with bony disease or testicular involvement do not appear to profit from radiation therapy to these sites. [215,316,317,318 and 319]

In light of these data and taking into consideration the acute toxicities, particularly injuries to skin and mucous membranes (worse in the presence of doxorubicin and MTX), as well as the late effects of radiation, including second malignancies, [304,320] it can be confidently stated that radiation has no *routine* role in the treatment of children with NHL, and its use *in any circumstance*, not excluding emergency therapy, must be clearly justified.

Similarly, surgery, although important to diagnosis and, where indicated, in dealing with the complication of therapy, has no other defined role in the treatment of childhood NHL when a highly effective, modern chemotherapy protocol is used. In few patients with LL is surgery even an option, and the potential for benefit lies largely in patients with abdominal tumor, the majority of whom has BL, BLL, or LBCL—that is, B-cell lymphoma. Early studies in African BL suggested that complete resection of bulky abdominal BL (readily accomplished in patients with, for example, isolated ovarian tumor, even if bulky) before chemotherapy was beneficial to survival.[40] It has been suggested that any apparent benefit of surgery, however, at least in sporadic BL/BLL is more a function of the smaller size, in general, of resectable tumors, than to the fact that the tumor has been completely excised. Retrospective studies in patients treated in the 1980s in the United States or Europe have shown that complete resection is much more likely to be accomplished in those presenting with an acute abdomen than in patients undergoing elective surgery who tend to have more extensive disease.[41,42] This is because the primary causes of an acute abdomen in patients with B-cell intraabdominal tumor are intussusception, which is caused by a small tumor partially projecting into the bowel lumen and "appendicitis" caused by occlusion of the appendiceal lumen by, again, a small volume of tumor. In view of this, it is not surprising that the treatment outcome in these retrospective studies was better in patients in whom all disease had been resected, because this group would include patients with smaller tumor bulk, many of whom presented with a surgical emergency. In the absence of a controlled study, however, it is not possible to determine the role of the surgical resection itself, and indeed, it may vary according to the chemotherapy subsequently instituted. Today, even patients with extensive intraabdominal disease, which would not be amenable to resection, enjoy an excellent prognosis with the most successful chemotherapy regimens so that there is no reason to advocate elective surgical resection. However, the original study of surgical resection conducted in Uganda [40] does suggest that in patients receiving what might today be considered minimal therapy (e.g., two standard doses of cyclophosphamide), a marked reduction in tumor burden is beneficial. In this study, although numbers were small, patients in whom only one of two massively involved ovaries were resected (by choice, to avoid sterilizing the patient) had a worse outcome than patients in whom both involved ovaries were resected. It remains possible, therefore, that in circumstances in which modern intensive chemotherapy cannot be given (e.g., in some developing countries), complete surgical resection may provide therapeutic benefit.

There appear to be no grounds for believing that surgical resection will reduce the low risk of bleeding or perforation associated with gastrointestinal tumor. In fact, the reverse is the case; in addition to the potential for delaying the institution of chemotherapy, there is a significant risk of fistula or perforation occurring in the postoperative period, not infrequently when the patient is granulocytopenic. [294]

The role of "second look" laparotomy has also been studied in B-cell lymphomas, particularly by the BFM group. [42,314] Because patients with viable tumor at the time of second look surgery did poorly regardless of the treatment modality used (usually, if localized, complete extirpation or radiation therapy), whereas those with no demonstrable viable tumor did well, second look surgery with or without complete resection cannot be recommended. Doubtless this is a consequence of the fact that B-cell lymphomas are disseminated from the outset such that evident residual disease represents only the tip of the iceberg and cannot be successfully treated by local therapy alone. Indeed, in patients treated with a very intensive regimen such as that used by the BFM group, patients in whom complete remission was not achieved had a very poor prognosis regardless of the salvage treatment used. It seems that the identification of residual tumor late in the course of therapy is unlikely to be of major value because therapeutic options are by then limited. On the other hand, the identification of slow responders very early in therapy, particularly if such patients are in what would otherwise be considered a low or intermediate risk group, may be beneficial because there is an opportunity to give such patients much more intensive therapy.[314]

**Specific Therapy**

Treatment protocols in childhood NHL, as with all treatment protocols, are designed to maximize benefit and minimize toxicity. Because patients with smaller tumor burdens, that is, at lower risk, require less "intensive" therapy, where *intensity* refers both to the number of milligrams of drug delivered per unit time and to the number of drugs incorporated into the regimen, it is critically important to identify the risk group into which the patient falls in order to tailor therapy accordingly. This process is generally referred to as *risk adaptation*. Any of the prognostic factors mentioned in the sections on staging and risk assignment may be used in this process of assigning patients to a specific treatment group, but in the end, the best indicator of the ultimate outcome of therapy is the response to treatment itself, such that a measure of the response to therapy in the early treatment cycles can be used, and has been successfully used, to identify patients who may benefit from more intensive therapy than that which their risk group dictates. The SFOP, for example, has used response to the prephase element, that is, the first week of treatment, as a factor in determining subsequent therapy.[302]

All childhood NHLs respond to a wide range of chemotherapeutic agents, partly because of their high growth fraction. Results in recent years using combination chemotherapy regimens have improved to the point that the most effective protocols are producing overall survival rates in the region of 90% for BL, BLL, and LBCL (70% to 90% for Burkitt's cell leukemia) and 80% to 90% for LL and ALCL. [26,255,264,265,283,302,305,314,315,321,322,323,324,325 and 326] It has become widely accepted that patients with B-cell lymphomas—namely, BL, BLL, and LBCL—require intensive, short-duration (3 to 6 months) therapy with multiple drugs, whereas those with LL are optimally treated with chemotherapy protocols based on the therapy of ALL. Patients with ALCL are usually treated with protocols identical to, or slightly modified from, those used for the therapy of B-cell lymphomas.

In pediatric NHLs, prophylaxis against the spread of tumor to the CNS is an essential component of therapy for the majority of patients. At the present time, the only patient groups that do not routinely receive prophylaxis against CNS disease are those with minimal disease, for example, patients with intraabdominal B-cell lymphomas that are completely resected, or patients with all histologies and stage I disease that is not in proximity to the CNS (i.e., not in the head and neck region or epidural), in whom CNS spread is a extremely rare. In a CCSG study, for example, none of 55 patients with resected abdominal disease treated with COMP developed a CNS recurrence.[34,327] Similarly, the SFOP LMB 89 protocol does not include any CNS prophylaxis for patients with stage I or II completely resected B-cell lymphoma, and no cases of isolated CNS relapse in this group have yet been observed. [302] However, isolated cases of CNS recurrence after complete resection of all abdominal tumor (B cell) have been occasionally observed, and because of the minimal increase in toxicity associated with the administration of a small number of doses of intrathecal MTX or Ara-C (e.g., four to six), some protocols do not omit CNS prophylaxis, even for this group. The balance here is the very small potential benefit weighed against the very small risk of severe neurotoxicity, such as myelopathy, that may occur after intrathecal therapy.

It is, of course, possible, that certain specific entities, for example, ALCL or subtypes of LBCL that have a much lower tendency to spread to the CNS than do BL or LL, do not require CNS prophylactic therapy, but this has yet to be shown in children, and unless demonstrated by carefully conducted clinical trials to be unnecessary, the majority of patients should continue to receive CNS therapy because the potential for harm (i.e., severe neurotoxicity) would appear to be greatly outweighed by the risk of the development of CNS recurrence if such therapy is not given. Intrathecal therapy with either MTX alone or MTX and Ara-C is usually the mainstay of CNS prophylactic therapy, but in patients with extensive disease, high-dose intravenous infusions of S-phase specific agents such as MTX and Ara-C are administered in the most effective protocols, whether for BL, BLL, and LBCL or for LL. Such agents probably benefit the patient both because of their effect against systemic tumor and because of their role in preventing CNS spread or dealing with occult CNS involvement.

Radiation of the cranium or craniospinal axis is generally not considered to have an advantage over chemotherapeutic CNS prophylactic therapy in children with NHL, and in some studies in patients with B-cell lymphomas, it has been shown to be ineffective. [329,330] Moreover, prophylactic cranial irradiation in ALL, in which there is considerably more experience with this approach, has been associated with significant toxicity, including impaired growth, intellectual impairment, and secondary brain tumors.[331,332,333,334,335 and 336] However, cranial radiation has continued to be used by the SFOP for the treatment of patients with CNS disease at presentation and as preventative therapy for patients with LL in the BFM and United Kingdom CCSG groups. [302,326,337] The value of radiation in these situations is currently being studied by randomized clinical trials.

***Chemotherapy of B-Cell Lymphomas***

For treatment purposes, children and adolescents with B-cell lymphomas (BL, BLL, and LBCL) may be considered together because there is no convincing evidence that, within this group of lymphomas, histology is an important determinant of outcome. Even subgroups of large cell lymphomas, for example, mediastinal LBCL, although comprising a small subset of most protocols, appear to have a similar outcome. Consequently, most cooperative groups use the same protocol for the full histologic spectrum of B-cell lymphomas. This has the major advantage that the treatment regimen employed is not dependent on the arbitrary and poorly reproducible separation of these entities on histologic grounds. In the B-cell lymphomas, short-duration, intensive therapy has clearly been shown to be superior to less intensive but longer duration treatment. Prolonging therapy beyond a few months at most (and a few weeks in patients with limited disease) appears to have no value, although longer therapy durations clearly have a potential for increased toxicity (early or late), increased expense, and increased inconvenience to the patient. There is no doubt that treatment protocols based on the principles used effectively for ALL, such as the LSA $_2$L$_2$ or the BFM 1976–81 regimens (which are several

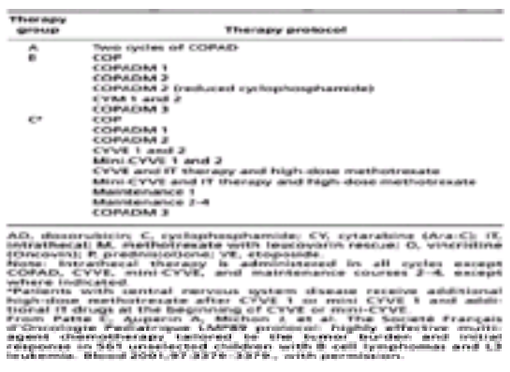
years in duration) are suboptimal for the treatment of patients with BL.[309,310,327] Even patients who present with diffuse bone marrow involvement, whether or not there is a high peripheral white count, have a poor prognosis with conventional therapy for ALL and a much better prognosis when treated with a protocol designed for B-cell lymphomas.[302,322,323,338,340]

The basic four-drug regimens used in the 1970s, consisting of cyclophosphamide, vincristine, prednisone, and MTX (generally low or intermediate dose) or doxorubicin, although still providing effective therapy for patients with minimal disease, cure only 50% to 75% of patients with more extensive disease.[310,324,327,340,341] A regimen known as APO [doxorubicin (Adriamycin), prednisone, vincristine (Oncovin)], based on ALL therapy, has met with similar success in large cell lymphoma in general, but this is probably because both B and T (predominantly ALCL, which can be reasonably well managed with ALL therapy, see below) have been included in series of patients treated with this regimen.[343] For patients with extensive disease, the basic drugs have been supplemented with higher dose MTX (5 to 8 g per $m^2$) and, frequently, high-dose Ara-C as well, in addition to other drugs, such as etoposide and sometimes ifosfamide.[302,314,315,323,328] These added drugs, higher dosages, or drug combinations were first shown to be effective in patients with recurrent disease.[344,345,346,347 and 348] Variations in individual and cumulative drug doses, timing, and total treatment duration exist among the several protocols in use, and present emphasis is on “fine tuning,” that is, refining drug dosage and administration. The optimal duration of MTX infusions is a major study question in the present BFM trial for B-cell lymphomas, whereas the French-American-British protocol for B-cell lymphomas, based on SFOP 89, is designed to determine whether reduction in dosage and in the total duration of therapy for specific patient subgroups can be safely achieved. In these multidrug protocols, the relative value of each drug cannot be determined, and in this respect it is of interest that corticosteroids are not included in the NCI protocol,[315,323] which appears to be as effective as SFOP and BFM protocols, both of which include corticosteroids, whereas anthracyclines have been shown in an as-yet unpublished, randomized study conducted by the CCSG to add no therapeutic value to the four-drug regimen, COMP. Because anthracyclines have the potential for acute or late cardiac toxicity[304] and certainly add to the acute toxicity of combination regimens (particularly with respect to myelosuppression and stomatitis), further studies to determine their utility in the present day 6 to 8 drug regimens may be warranted.

To minimize the possibility of tumor regrowth between treatment cycles and to achieve high-dose intensity, sequential cycles are usually begun as soon as there has been sufficient recovery from the toxicity of the previous cycle—usually when the granulocyte count reaches 500 or 1,000 per $mm^3$. Toxicity is minimized by adjusting the intensity of therapy to the risk category into which the patient falls. This process of patient stratification by risk group is one of the most important aspects of modern protocol design. Inappropriate assignment could lead either to some patients receiving less therapy than they require, with a resultant worse outcome for these patients, or to some patients receiving more therapy than they require, with a greater risk of toxicity than necessary. Since toxicity even with the most intensive therapies is acceptable, however, if there is doubt as to which group a patient fits into, it is reasonable to opt for the higher risk treatment arm. It is necessarily the case that many patients, such as that fraction of patients with extensive disease who would have been cured with one of the earlier four-drug regimens, do in fact receive more therapy than they require. In the absence of a more accurate means to identify such patients, there is no other alternative.

It has become clear that stage is an insufficient means to stratify patients for therapy. Occasional patients with stage I disease have quite extensive tumor, for example, a localized mass of more than 10 cm, and although there is insufficient published data on which to draw solid conclusions, it would seem that such patients ought to be included in a higher risk category than would be the case if stage alone were the determinant of therapy. Serum LDH levels are used by the BFM group as a determinant of risk group based on their earlier finding that stage III patients with a serum LDH higher than 500 U per L had approximately double the survival rate when the MTX dosage was increased from 0.5 g per $m^2$ to 5 g per $m^2$.[283]

The results of several protocols in which the principle of risk stratification is used demonstrate that excellent results can be achieved, overall approximately 90% event-free survival (EFS) (Table 24-11, Table 24-12, Table 24-13, and 24-14). In particular the SFOP LMB 89 and the German BFM 90 protocols have been used in more than 1,000 patients each, with EFS rates of more than 90% for all patients, 76% in patients with the most extensive disease (leukemic patients) treated on BFM 90, and 98% to 100% for stage I and II patients.[283,302] Comparable results have been obtained by the U.S. NCI protocol 89-C-41, but the number of patients accrued is much smaller.[315,323] The U.S. Pediatric Oncology Group (POG) results are also not greatly different ( Table 24-15). In each of these protocols, the significance of prognostic factors has largely been lost. Even patients with stage IV disease do not have a significantly worse prognosis, although CNS disease may still be associated with a somewhat worse outlook. Thus, at this point, the major questions to be asked in the treatment of these diseases is whether equally good results can be achieved with less toxicity and whether further improvements in risk stratification can be made. A means of identifying precisely patients destined not to achieve complete remission could permit even more intensive therapy or perhaps the substitution of drugs, particularly in the event that resistance to one or more of the agents in the planned protocol could be identified in advance. It is possible that newer techniques, such as microarray analysis or proteomic analysis of gene expression patterns, or methods of measuring more precisely the rate of response of tumors to therapy, could lead to such precision.



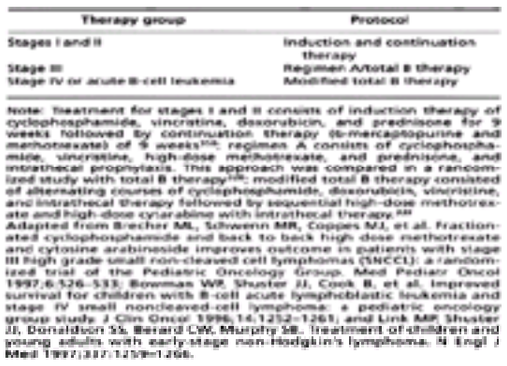
| Therapy group | Therapy protocol |
|---|---|
| A | Two cycles of COPAD |
| B | COP |
| | COPADM 1 |
| | COPADM 2 |
| | COPADM 2 (reduced cyclophosphamide) |
| | CYM 1 and 2 |
| | COPADM 3 |
| C* | COP |
| | COPADM 1 |
| | COPADM 2 |
| | CYVE 1 and 2 |
| | Mini CYVE 1 and 2 |
| | CYVE and IT therapy and high-dose methotrexate |
| | Mini CYVE and IT therapy and high-dose methotrexate |
| | Maintenance 1 |
| | Maintenance 2-4 |
| | COPADM 3 |

AD, doxorubicin; C, cyclophosphamide; CY, cytarabine (Ara-C); IT, intrathecal; M, methotrexate with leucovorin rescue; O, vincristine (Oncovin); P, prednisone(ne); VE, etoposide.
Note: Intrathecal therapy is administered in all cycles except COPAD, CYVE, mini CYVE, and maintenance courses 2–4, except where indicated.
*Patients with central nervous system disease receive additional high-dose methotrexate after CYVE 1 or mini CYVE 1 and additional IT drugs at the beginning of CYVE or mini-CYVE.
From Patte C, Auperin A, Michon J, et al: The Societe Francais d'Oncologie Pediatrique LMB89 protocol: highly effective multiagent chemotherapy tailored to the tumor burden and initial response in 561 unselected children with B-cell lymphomas and L3 leukemia. Blood 2001;97:3370–3379., with permission.

**TABLE 24-11. OUTLINE OF THERAPY IN PROTOCOL FRENCH-AMERICAN-BRITISH LYMPHOME MALIGNE 96 FOR B-CELL LYMPHOMAS**

| | |
|---|---|
| **Lymphoblastic lymphoma** | |
| Low-risk group | Protocol I—protocol M—maintenance therapy |
| Intermediate-risk group | Protocol I—protocol M—protocol II—maintenance therapy |
| High-risk group | Treatment according to high-risk arm of ALL-BFM-90 or NHL-BFM-90 |
| **Burkitt's and large B-cell lymphomas** | |
| Risk group 1 | Cycles A and B (no prophase and vincristine is omitted) |
| Risk group 2 | Prophase followed by four cycles: A-B-A-B |
| Risk group 3 | Prophase followed by five cycles: AA-BB-CC-AA-BB |
| Risk group 4 | Prophase followed by six cycles: AA-BB-CC-AA-BB-CC |
| **Anaplastic large cell lymphoma** | |
| Stage I, II resected | Prophase followed by three cycles: A-B-A |
| Stage II not resected and III | Prophase followed by six cycles: A-B-A-B-A-B |
| Stage IV | Prophase followed by six cycles: AA-BB-CC-AA-BB-CC |

**TABLE 24-12. OUTLINE OF THERAPY IN PROTOCOL BERLIN-FRANKFURT-MüNSTER–NON-HODGKIN'S LYMPHOMA 90**



| Therapy group | Protocol |
|---|---|
| Stages I and II | Induction and continuation therapy |
| Stage III | Regimen A/total B therapy |
| Stage IV or acute B-cell leukemia | Modified total B therapy |

Note: Treatment for stages I and II consists of induction therapy of cyclophosphamide, vincristine, doxorubicin, and prednisone for 9 weeks followed by continuation therapy (6-mercaptopurine and methotrexate) of 9 weeks[336]; regimen A consists of cyclophosphamide, vincristine, high-dose methotrexate, and prednisone, and intrathecal prophylaxis. This approach was compared in a randomized study with total B therapy[336]; modified total B therapy consisted of alternating courses of cyclophosphamide, doxorubicin, vincristine, and intrathecal therapy followed by sequential high-dose methotrexate and high-dose cytarabine with intrathecal therapy.[335]
Adapted from Bricher ML, Schwenn MR, Coppes MJ, et al. fractionated cyclophosphamide and back to back high dose methotrexate and cytosine arabinoside improves outcome in patients with stage III high grade small non-cleaved cell lymphomas (SNCCL): a randomized trial of the Pediatric Oncology Group. Med Pediatr Oncol 1997;6:126–133; Bowman WP, Shuster JJ, Cook B, et al. Improved survival for children with B-cell acute lymphoblastic leukemia and stage IV small noncleaved-cell lymphoma: a pediatric oncology group study. J Clin Oncol 1996;14:1252–1261; and Link MP, Shuster JJ, Donaldson SS, Berard CW, Murphy SB. Treatment of children and young adults with early-stage non-Hodgkin's lymphoma. N Engl J Med 1997;337:1259–1266.

**TABLE 24-13. OUTLINE OF THERAPY FOR PEDIATRIC ONCOLOGY GROUP PROTOCOLS FOR PATIENTS WITH BURKITT'S LYMPHOMA OR BURKITT'S-LIKE LYMPHOMA**

| Therapy group | Protocol |
|---|---|
| Low-risk patients | Three A cycles |
| High-risk patients | Four cycles: A-B-A-B |

A, cyclophosphamide, doxorubicin, vincristine, and high-dose methotrexate; B, high-dose cytarabine, ifosfamide, and etoposide. Adapted from Magrath I, Adde M, Shad A, et al. Adults and children with small non-cleaved cell lymphoma have a similar excellent outcome when treated with the same chemotherapy regimen. J Clin Oncol 1996;13:925–935; and Adde M, Shad A, Venzon D, et al. Additional chemotherapy agents improve treatment outcome for children and adults with advanced B-cell lymphomas. Semin Oncol 1998;2[Suppl 4]:33–39.

**TABLE 24-14. OUTLINE OF THERAPY IN U.S. NATIONAL CANCER INSTITUTE PROTOCOL 89-C-41 FOR B-CELL LYMPHOMAS**

| | No. patients | Event-free survival at 3 yr or more (%) |
|---|---|---|
| **LMB 89**[352] | | |
| Stage I and II patients | 122 | 96±4 |
| Stage III patients | 280 | 93±3 |
| Stage IV | 97 | 93±4 |
| Leukemic patients | 67 | 79±8 |
| **Berlin-Frankfurt-Münster 90**[353] | | |
| Stage I | 49 | 91±5 |
| Stage II | 114 | 98±1 |
| Stage III | 171 | 86±3 |
| Stage IV | 23 | 81±8 |
| Leukemic patients | 56 | 76±8 |
| **Pediatric Oncology Group protocols** | | |
| Stage I and II[358] | >100 | 88 |
| Stage III* | 64 | 79±6 |
| Stage IV[326] | 55 | 79±9 |
| Leukemic patients[326] | 74 | 65±8 |
| **U.S. National Cancer Institute 89-C-41** | | |
| Low-risk patients[355] | 55 | 100 |
| High-risk patients[327] | 66 | 85 |

*Results given are those for the modified total therapy III protocol.[326]

**TABLE 24-15. RESULTS OF VARIOUS PROTOCOLS IN THE TREATMENT OF B-CELL LYMPHOMAS**

Patients with CNS disease have long been considered to have a particularly poor prognosis. There are several possible explanations for this. Such patients could have biologically different disease, which is more resistant to the particular chemotherapy in use; CNS disease may be associated with particularly extensive systemic disease; or the therapy used is less effective, for reasons of drug distribution, against CNS disease. Of interest in this context is the observation, published many years ago, that African patients with BL and CNS disease did not have a worse prognosis than other patients. Indeed, even with the therapy delivered in the 1960s and 1970s, often single-agent therapy, 50% could be expected to achieve long-term survival. [236,349] This is probably related to the fact that in endemic BL, CNS disease is frequently associated with rather small tumor burdens. In contrast, CNS disease in sporadic BL is associated with extensive systemic disease, frequently including bone marrow involvement.[218,226,227] Based on evidence from two different series, it seems probable that CNS disease in sporadic BL previously had a poor prognosis because of its association with a high tumor burden, perhaps coupled to the earlier, inadequate therapeutic approach to CNS disease. [218,226] The use of high-dose S-phase agents—that is, MTX and Ara-C, both of which are highly active in BL, and which provide effective drug levels in the spinal fluid and CNS parenchyma—has dramatically improved the results of therapy in patients with CNS disease. Both high-dose Ara-C and high-dose MTX are potential neurotoxins, and this must be taken into consideration in protocol design because neurotoxicity may be considerably increased if other potential neurotoxins are used. [350] This is an additional reason to avoid the use of radiation in patients with CNS disease or those at risk of developing it.

With the improved outcome of patients with B-cell lymphomas, an interesting change in the pattern of failure has occurred. Most failures, although few (approximately 10% of patients), are now caused by primary resistance to therapy, that is, the patient achieves only a partial response, or by toxic death. Recurrent disease has become rare and is almost never observed beyond 1 year after the initiation of therapy. Patients who remain in their first disease-free remission at this time may, as has always been the case, be considered cured. Late relapse (beyond 1 year) has been reported only in a small percentage of African patients with BL; in such patients there is evidence that “relapse” is, in reality, a new neoplasm. [351] Presumably, this results from the significantly higher risk in African children of developing BL, but it may also indicate the presence of genetic factors that markedly increase the risk of developing BL in specific individuals. The identification of familial cases of BL provides some support for the influence of a genetic factor of this kind. [59,352,353] This observation has a parallel in acquired immunodeficiency syndrome patients, also at particularly high risk for the development of B-cell neoplasms, in whom “late relapse” has been shown to be caused by the development of a clonally distinct neoplasm rather than recurrence of the original clone. [354]

***Chemotherapy of Lymphoblastic Lymphoma***

In the randomized trial completed by the Children's Cancer Group more than 15 years ago in which ALL-type therapy ($LSA_2L_2$) was compared with the COMP regimen for all NHLs, the $LSA_2L_2$ regimen, although clearly inferior for non-LLs, proved to be superior for LL. [327] Consequently, because the results of this study became widely know, the treatment of LL has been largely limited either to variants of $LSA_2L_2$ itself or to other treatment protocols designed for ALL. Such protocols include induction, consolidation, and maintenance elements lasting for a total of between 1 and 3 years. [325,326,337,355,356,] Although regimens similar to protocols used for patients with B-cell lymphomas have been used in LL and have resulted in the cure of a reasonably high fraction of patients, [342,355] the results of these protocols in patients with bone marrow disease were quite poor. It is possible that more intensified versions of these protocols, such as those used for B-cell lymphomas (perhaps followed by a maintenance therapy), would achieve better survival rates, but results in LL with therapies used for ALL are sufficiently good and sufficiently well tolerated that there is little incentive to test alternative treatment approaches. The best reported results to date are those of the BFM group, which has achieved an estimated EFS rate at 5 years of 92% for patients with T-cell LL. [326] It is important to note that these excellent results have been obtained without the use of local radiation.

In LL, patients with limited disease (localized or multiple disease sites outside the thorax and on one side of the diaphragm) as well as patients with stage IV disease (i.e., CNS disease or less than 25% of blasts in the bone marrow—with a higher percentage, patients would be considered to have ALL) are uncommon. Thus, many investigators have divided LL into two risk groups, limited (stages I and II) or extensive (stages III and IV), or have not divided them at all and used the same therapy for all patients. In part this is because the infrequency of patients with limited disease has led to a lack of information with respect to the most appropriate therapy to use. It would seem logical to base therapy on that used for precursor B-cell ALL, because LL patients with limited disease more often have LL that is immunophenotypically identical to precursor B-cell ALL. The ability to detect bone marrow involvement by flow cytometry in some of these patients (in whom the bone marrow is generally microscopically normal) suggests that such cases are unusual manifestations of ALL. In the CCSG study conducted with $LSA_2L_2$, children with limited-stage LL had an identical outcome (approximately 80% long-term survival) whether treated with $LSA_2L_2$ or with COMP, although there were only 11 such patients,[327] which is similar to the result that might have been expected in ALL. In this trial COMP was given for 18 months, and the study included local irradiation. A subsequent study demonstrated that 6 months of therapy was as effective as 18 months in patients with limited-stage disease. [357] Using a shorter duration of therapy [9 weeks of CHOP (cyclophosphamide, hydroxydaunomycin, vincristine [Oncovin], prednisone), with or without local radiation], POG reported approximately 70% EFS rates using the same treatment approach as that used in patients with limited B-cell NHL. [39,358] This result would appear to be unsatisfactory when compared to results of 80% to 90% being achieved today in patients with stage III disease, but whether the lower survival rate is due to differences in the drug combinations (e.g., lack of MTX, asparaginase, or Ara-C in the treatment regimen) or to an inadequate duration of therapy is unknown. In protocol LMT81 used at the Institut Goustave Roussy,[325] which included ten administrations of high-dose MTX in conjunction with the $LSA_2L_2$ protocol, there were only eight stage I and II patients, and two died in

remission from measles (all the remaining patients achieved long-term survival). No meaningful conclusion can be drawn from this small number of patients as to the added advantage of the infusions of high dose MTX in patients with limited disease. Similarly, in the BFM-NHL 86 protocol, there were only six evaluable patients with limited-stage (I or II) disease out of a total of 73 patients with LL treated with BFM protocols for ALL (two relapsed). [314] In the more recent BFM 90 protocol, there were four patients with T-cell LL, none of whom relapsed.[326]

At the present time, although it is clear that a high fraction of patients with limited-stage disease can be cured, optimal therapy has not been identified. Based on small numbers, it would appear probable that short-duration therapy is inadequate, although it is unclear whether ALL-type induction is superior to CHOP- or COMP-type inductions. Nor is it known whether precursor T-cell LL requires different therapy from precursor B-cell LL. The answers to these questions will only be obtained by conducting clinical trials involving patients from several countries.

Although LL can be effectively treated using ALL protocols, not all such treatment protocols are equally satisfactory. Some years ago, T-cell ALL had a significantly worse prognosis using the protocols then extant, and because LL is predominantly of T-cell immunophenotype, these earlier protocols may be inadequate for LL as well. The St. Jude Study VIII protocol, for example, produced very poor results in LL, with only 10% of patients surviving at 2 years. [359] $LSA_2L_2$ itself has produced long-term EFS rates of only 64% for patients with disseminated disease in the Children's Cancer Group protocol [327] and less than 60% when modified slightly and used by POG.[355] These results would seem to have been improved by the addition of ten administrations of high-dose MTX by the group at Goustave Roussy, [325] which reported an EFS at 5 years of 78% in 84 patients, 79% for stage III patients, and 72% for stage IV patients. Because randomized comparison was not performed, the conclusion that high-dose MTX adds therapeutic efficacy to $LSA_2L_2$ must be considered tentative. Although the BFM 90 protocol was also a single arm study, the EFS rate of 92% at 5 years in 101 evaluable patients and no significant difference between stage III (91%) and stage IV (94%) patients is considerably greater, suggesting that the protocol is truly superior to $LSA_2L_2$. This will become apparent if the results of the next BFM protocol are similar.

BFM 90 consisted of relatively intensive initial therapy lasting 30 weeks that, except for patients with limited-stage disease, included two “induction” regimens separated by four cycles of high-dose MTX. The “late” intensification, in fact a repeat induction cycle, has been shown to be an important component of this protocol when used for the treatment of ALL,[360] and it improves EFS rates even in patients with good-risk ALL.[361] Asparaginase is likely also to be an important component of this regimen, and the POG recently showed that repeated doses of high-dose asparaginase (25,000 IU per $m^2$ intramuscularly weekly for 20 weeks) early in therapy improved the outcome for patients with LL from 64% to 78% continuous complete remission at 4 years.[362] CNS prophylaxis in BFM 90 included intrathecal therapy, high-dose MTX, and radiation therapy. The value of the radiation therapy, as alluded to earlier, is questionable in this context, and its use is being formally examined in the successor study, BFM 96.

Patients with LL who have not relapsed after 30 months from the start of treatment have a very high probability of remaining in complete remission and in fact, no relapses were observed after 12 months from diagnosis in the BFM 90 protocol. [326] With so few relapses, it is perhaps not surprising that prognostic factors could not be identified. Even the rate of response to therapy was not predictive of outcome, although the rate of response was an important prognostic factor in a United Kingdom CCSG study.[363] Differences of this type may related to differences in the patient population or the treatment used, or may arise by chance.

***Chemotherapy of Anaplastic Large Cell Lymphomas***

ALCL is a relatively recently recognized entity, and as such, optimal therapy has yet to be defined. Reasonably good results have been obtained with a variety of regimens, whether treatment strategies have been based on treatment designed for ALL/LL or for B-cell lymphomas. An Italian group, for example, used $LSA_2L_2$ and, in some patients, local irradiation and observed 5-year survival and progression-free survival of 84% and 72%, respectively. [364] Similar results have been obtained by the SFOP using a protocol based on its successful therapy for B-cell lymphomas. In the latter protocol, two cycles of COPADM [cyclophosphamide, vincristine (Oncovin), prednisone, doxorubicin (Adriamycin), MTX] were followed by 5 to 7 months of maintenance therapy. [365] Seventy-eight patients out of 82 (95%) achieved a complete remission and 21 relapsed. Survival and EFS at 3 years were 83% and 66% respectively, with a median follow-up of 49 months. This result, although promising, appears to be poorer than that achieved in patients with LBCL, as has also been reported by the POG, [366] but in the case of the SFOP, at least, the therapy delivered was less intensive than that delivered for B-cell lymphomas. The BFM group has also demonstrated the efficacy, in ALCL, of the type of therapy designed for B-cell lymphomas. Patients with ALCL were enrolled onto successive BFM protocols that specified a slightly modified version of the standard protocol for B-cell lymphomas.[265,324] Survival and EFS probabilities at 9 years were both in excess of 80% and, unlike the French series, there was no significant difference between survival and EFS. Skin and mediastinal involvement appeared to be negative prognostic factors in the first 62 patients, [265] but these factors could not be shown to be significant prognostic factors when more patients were accrued.[324] The BFM results appear to be among the best reported in children to date, and this protocol will be adopted more widely in Europe in the future.

ALCL has also been shown to respond well to the ABVD [doxorubicin (Adriamycin), bleomycin, vinblastine, dacarbazine] regimen, a protocol used widely for the treatment of Hodgkin's disease. In a randomized study conducted in a small number of patients (40), ABVD was compared to MACOP-B [MTX-leucovorin, doxorubicin (Adriamycin), cyclophosphamide, vincristine (Oncovin), prednisone, bleomycin], a treatment regimen used in adult patients with “diffuse aggressive lymphomas.” Similar results were obtained with both treatment approaches[367]: 90% of patients treated with MACOP-B and 91% of those treated with ABVD achieved a complete response. The probability of relapse-free survival, projected to 32 months, was 94% for patients treated with MACOP-B and 91% for those treated with ABVD. These results must be considered as preliminary because of the small size of each arm of the study.

In summary, there is no doubt that a variety of regimens can be successfully used for the treatment of ALCL, but at the present time therapeutic protocols designed for B-cell lymphomas are the most widely used in children. Stratification for treatment arms has generally been similar to that used in B-cell lymphomas, although patients with bone marrow and CNS disease are uncommon. There is also some evidence that ALK-positive ALCL, which tends to occur more often in children and young adults, has a better treatment outcome than ALK-negative ALCL.[368] The level of soluble CD30 antigen in serum is also correlated negatively with survival, possibly because it binds CD30 ligand, preventing it from interacting with CD30, a receptor that is a member of the tumor necrosis family of receptors involved in the induction of apoptosis.[369]

**Treatment after Partial Response or Relapse**

Partial response, in the absence of clear-cut regrowth of tumor, should be documented by biopsy because of the frequent persistence of abnormalities due to non-viable tumor on imaging studies. Gallium-67 or positron emission scintigraphy may help in determining whether residual abnormalities are composed of viable tumor, but such studies, too, are not specific. True partial response indicates that the tumor is primarily resistant to the chemotherapy regimen being used; it follows that if all elements of the planned regimen have been employed immediately before a partial response is declared, then continuation with the same therapy is unlikely to be successful. The options for further therapy, to a degree, depend on the number of drugs and the doses used up to that point, and on the histologic subcategory of the disease. Because primary regimens have been changing, and many drugs that were originally used in salvage regimens have now been brought into primary therapeutic protocols with resultant higher cure rates, the approach to the management of the small number of patients in whom primary therapy does not result in cure must also change, and it is to be anticipated that the results of therapy in such patients is likely to be very poor.

***B-Cell Lymphomas***

In patients with B-cell lymphomas, partial response has become a more frequent cause of failure than recurrent disease after a period of complete remission. Because relapses, if they do occur, do so (with rare exceptions) 6 to 8 months after the cessation of therapy, it is also likely that the recurrent tumors are chemotherapy resistant, differing perhaps only with respect to the proportion of primarily resistant cells present at the time of commencement of therapy. As such, in the absence of treatment with non–cross-resistant therapy, it is unlikely that a significant response (i.e., more than a transient response) will be achieved. In general, radiotherapy is not helpful in these patients, primarily because it is not a very effective modality (see above) but also because patients usually have disseminated disease at the time of relapse. An approach that has been quite successful in the past, particularly with less intensive primary therapy, is the use of high-dose therapy with stem cell rescue, usually autologous bone marrow transplantation (ABMT). ABMT was developed at the NCI using BACT (bischloroethylnitrosourea, Ara-C, cyclophosphamide, 6-thioguanine), a regimen that had been used to prepare patients with aplastic anemia for allogeneic transplantation. [370] In this first series, 4 of 19 patients were cured after relatively nonintensive cyclophosphamide-containing regimens. BACT was modified by Philip and colleagues by the substitution of etoposide for 6-thioguanine (BEAC) or etoposide and melphalan for 6-thioguanine and cyclophosphamide (BEAM). [371,372] This approach has subsequently been shown to be effective only in patients with documented chemotherapy-sensitive tumors—that is, patients in whom a second regimen produces a good response—and to be of no benefit to patients with primary refractory disease or resistant relapse. [371,372,373 and 374] Consequently, the usual approach is to first treat patients in whom only partial response is achieved or in whom relapse occurs with a drug combination that includes drugs previously not used and selected in the hope that they will be non–cross-resistant. In patients

whose tumors respond well, additional high-dose therapy with stem cell rescue is given. In a recent report, in patients treated between 1979 and 1991, approximately one-third of patients who failed to achieve remission or who relapsed were salvaged by ABMT, although there were no survivors among patients who had primary refractory disease or resistant relapse.[374] As therapy has continued to evolve since this time, treatment failures (a smaller and smaller number) fall more and more into these two categories, whereas the results of salvage therapy, presumably because of the previous intensive therapy, become worse and worse. In addition, the small numbers of patients requiring salvage therapy make studies of the most effective therapy in such patients difficult. This difficulty, however, reflects the success of primary treatment regimens.

The choice of a salvage regimen is not easy. Because platinum compounds are generally not used in primary therapy, either cisplatin- or carboplatin-containing regimens are frequently used. A popular “salvage” regimen for a variety of tumors in recent years, including NHL in adults and children, has been ICE, a combination of ifosfamide, carboplatin, and etoposide,[375,376,377 and 378] although this regimen is unlikely to be effective in patients who have previously received full-dose ifosfamide and etoposide. ICE represents one of the combinations based on etoposide and ifosfamide that have been shown to be active agents in children and adults who have previously been unexposed to these drugs.[379] A number of ICE regimens, differing with respect to the doses of the constituent agents, have been used, some at sufficiently high dosage that stem cell rescue is required (or advisable), in which case, the regimen becomes one of repeated rounds of ICE and stem cell rescue rather than ICE followed by another high-dose regimen and ABMT. ICE has also been used with additional agents, for example, paclitaxel, with promising results,[380] and other platinum-containing regimens have also been used, particularly in adult patients with recurrent lymphoma.[381] The choice of a conditioning regimen for ABMT (if ICE itself is not used) is also difficult. The traditional regimen—high-dose cyclophosphamide with total body irradiation—has evolved into a broad range of regimens,[382] although few comparative studies of the pros and cons of these various protocols have been conducted.

Patients with isolated CNS relapse, including both African and U.S. patients may achieve long-term survival when treated with conventional systemic therapy and intrathecal chemotherapy.[28,218,227,236] Although bone marrow transplantation has sometimes been advocated in such patients, extrapolating from European Bone Marrow Transplant Lymphoma Registry data, which includes predominantly adult patients, the presence of active CNS disease at the time of ABMT was associated with a poor prognosis, although a small number of patients (probably less than 10%) may achieve prolonged disease-free survival. The presence of CNS involvement before ABMT did not adversely affect the outcome, so that CNS involvement at some time before high-dose therapy should not be considered a contraindication to ABMT.

Allogeneic bone marrow transplantation following high dose chemotherapy, with or without total body irradiation, has also been explored in relapsed patients with B-cell NHL, and overall results are quite similar to the ABMT experience, although data is much more limited.[383] The possibility of a graft-versus-tumor effect, based on the generally lower relapse rate with allogeneic rather than autologous transplant, makes this more attractive on theoretical grounds, and considerable effort has been expended in recent years on the identification of alternative sources of stem cells, including the use of haplotype mismatched donors, unrelated donors, cord blood cells, and positively selected stem cells, because of the limited availability of sibling donors and the complications of graft-versus-host disease.[384,385,386 and 387]

### *Lymphoblastic Lymphoma*

Because LL is generally treated with protocols closely modeled on therapy designed for ALL, recurrent disease is also usually managed on principles that have been largely derived from the management of recurrent ALL. As with B-cell lymphomas, partial responders have an extremely poor prognosis, as do patients who relapse on or shortly after the completion of therapy. In the latter case, standard (although intensive) reinduction therapy may be used if the patient has not received such therapy for some time, and such patients have a high chance of achieving remission, although the remission is likely to be of short duration. Alternatively, an intensive protocol using at least some drugs not previously received by the patient (e.g., ICE therapy) may be used. Patients who respond to such salvage therapy would be candidates for allogeneic bone marrow transplantation (if a sibling match is unavailable, a haplotype-mismatched parental graft, cadaveric graft, or autologous transplant may be considered). For patients who relapse off therapy (i.e., more than 6 months after the cessation of chemotherapy), reinduction with a similar regimen to that used before, followed by intensified consolidation and maintenance therapy is more likely to be successful. The role of transplantation in such patients is less clear and depends to a large extent on the duration of complete remission before relapse—the later it is, the better the prognosis. Although many would advocate bone marrow transplantation for patients who relapse “off therapy, too,” the longer the duration of remission before relapse, the more likely is conventional therapy to offer as effective an approach as bone marrow transplantation.[388 and 389]

### *Anaplastic Large Cell Lymphoma*

As noted above, many patients with ALCL who relapse respond well to a variety of salvage therapies. In perhaps the largest series of children with recurrent ALCL,[390] 28 patients received lomustine, vinblastine, and Ara-C, with or without bleomycin, and the remaining 13 patients received a variety of salvage therapies. Eighty-eight percent achieved a complete second remission. Fifteen of these patients underwent bone marrow transplant (autologous in 14); 44% remained disease free and 69% were survived at 3 years. ABMT in second remission appeared to provide no advantage. In 8 of 13 patients treated for a relapse (not necessarily the first relapse), prolonged remission was achieved with weekly vinblastine as the sole therapy (six of these patients had relapsed after ABMT). In other studies, some patients have achieved prolonged remission with *cis*-retinoic acid or *cis*-retinoic acid and interferon-a.[391,392] Thus, patients who relapse with ALCL should not automatically be referred for high-dose therapy with stem cell rescue. In fact, although such therapy has been successful, as demonstrated by the French experience, it is not clearly superior to other treatment approaches for patients with recurrent ALCL. This situation is reminiscent of African BL, in which a high proportion of long-term survivors have relapsed, sometimes on multiple occasions.[28,236] It is unclear why African BL and ALCL should differ with respect to the sensitivity of recurrent disease, and our concepts of the underlying biology or recurrent disease are challenged by these observations. It is also important to note that even patients who have more than one relapse may benefit from chemotherapy, even single-agent chemotherapy such as vincristine, *cis*-retinoic acid, or interferon-a.

## LONG-TERM COMPLICATIONS OF THERAPY

The long-term complications of the treatment of NHL in children have become less of a problem as treatment has been refined. The potentially serious consequences of radiation therapy on growth and development (including psychological development in patients undergoing cranial irradiation or bone marrow transplant[393]), on fertility, and as a potentiating factor in second malignancies have diminished as this modality has diminished in importance in therapeutic strategies.[304] The trend toward shorter therapy durations in the B-cell lymphomas will almost certainly further reduce the likelihood or seriousness of long-term complications relating to drug therapy, such as impaired reproductive function, the adverse effects of anthracyclines on the heart, and the risk of second malignancies. When combined-modality therapy (chemotherapy and radiotherapy) is used, the risk of many of these complications is significantly increased. Late cardiotoxicity relating to anthracyclines, however, has become an important problem as more patient survive long-term. It occurs at much lower cumulative doses than does acute cardiotoxicity—indeed, it has been suggested that there may not be a “safe level” at which no late cardiotoxicity occurs—and may affect as many as 25% of children with cancer treated with an anthracycline.[394]

Reproductive function is normally severely impaired in males, even prepubertal males undergoing chemotherapy, particularly when high doses of alkylating agents or radiation are used (even scatter from cranial irradiation may impair fertility[395,396 and 397]), but a significant fraction of patients retain the ability to reproduce. In women treated before age 20 years who do not receive abdominal irradiation, reproductive function later in life appears to be normal, and regimens based on acute leukemia therapy are relatively benign with regard to reproductive function, even in males,[396] although once again, due attention must be paid to the particular chemotherapy regimens used. In a recent study of reproduction after chemotherapy in young patients treated with regimens for non-LLs in use 10 to 25 years ago, the effect on reproductive function in both males and females appeared to be rather mild,[304] although precise fertility rates are difficult to obtain in small series of patients of varying age. Nonetheless, the available data serve to demonstrate that fertility can be retained (or returns) in a significant fraction of patients.

In contrast to the situation in Hodgkin's disease and the solid tumors of children, second malignancies have not been a significant problem in the majority of studies reporting on this complication in patients with NHLs,[304,398] although it is highly probable that the incidence of second neoplasms will vary with the specific protocol being used. This may be the explanation for a report from the United Kingdom in which a frequency of 8.6% of second malignancies was described. Six of the eight patients who developed a second tumor had received adjuvant radiotherapy.[399] Even secondary leukemias, often associated with 11q23 chromosomal abnormalities, which have been reported to occur relatively soon (usually within 2 years) after the administration of etoposide[400] do not appear to be a problem with modern regimens, presumably because of the dose and schedule of etoposide used.[304]

In all, late effects of treatment appear to be relatively mild in comparison, for example, with those encountered in Hodgkin's disease, and are likely to be fewer than in the past, as surgery and radiation are no longer primary treatment modalities. Nevertheless, the potential for late effects is a major consideration in the design of new

chemotherapy protocols, precisely because the results of modern therapy are so good.

## FUTURE CONSIDERATIONS

The treatment of NHLs in children has improved greatly in the last 15 years. The best reported results indicate that approximately 90% of all patients can be cured when treated optimally. Even the majority of patients in developing countries can be cured, although only in centers in which the cost of the drugs is met and there is reasonable supportive care. This result has largely been achieved by the refinement of chemotherapy protocols. The emphasis for the near future is on further refinement of risk groups such that patients are not over- or undertreated. It should be remembered, however, that overtreatment is deleterious only to the extent that it is toxic (whether acutely or in the long term) such that the risk of toxic side effects must be weighed against the risk of undertreatment, which has the tragic consequence of disease recurrence. Of particular importance is the identification of patients destined to do poorly so that they can receive more intensive or experimental therapies, preferably before disease progression has occurred. Because myelosuppression is the major acute toxicity, which can be associated with life-threatening infection, a number of investigators have explored the role of colony-stimulating factors in reducing these side effects. Although these agents clearly are effective at reducing the duration of neutropenia, there is less clear-cut information as to their value in reducing the risk of febrile neutropenia or overt infection. [323] The use of prophylactic antibiotics should be carefully researched in view of the ever-increasing risk of emerging resistant organisms, but in situations in which the majority of patients requires antibiotics because of fever, the risk of the development of resistant organisms, although real, may not be enhanced by this practice and could well reduce the in-hospital time and risk of septicemia. Indeed, there is considerable evidence from the bone marrow transplantation literature that patients undergoing intensive therapy and experiencing severe neutropenia can be largely treated as outpatients with judicious use of colony-stimulating factors and oral antibiotics.[401,402] Thrombopoietics (or combinations of colony-stimulating factors that influence megakaryocyte differentiation), although just beginning to be examined in the clinical setting,[403] are likely, at best, to lessen the need for platelet transfusions and will probably have little or no impact on toxic death rates. Thus, major progress is likely only to be made by the development of therapy that is more directly targeted to the tumor cell, such that side effects are minimized. Such approaches are presently being extensively studied and are made possible by the ever increasing knowledge of the molecular genetic and resultant biochemical abnormalities that underlie the pathogenesis of lymphoid neoplasms.[404] This information is also likely to be valuable to the improvement of diagnostic techniques and to the identification of prognostic subgroups. Highly specific (i.e., targeted) therapy is likely to be much less toxic and is likely to eventually replace conventional cytotoxic therapy, although the excellent results currently being obtained with the latter make it difficult to depart from accepted practice, and early trials are likely to include a combination of standard and targeted therapy.

Recently, promising results have been obtained with the use of monoclonal antibodies directed against B-cell antigens, particularly CD20, which is not modulated from the surface of tumor cells.[405,406] and [407] Such approaches appear to be much more successful in the more indolent lymphomas, but their use in targeted radiotherapy, including treatment of meningeal infiltration, [408] ombined with standard chemotherapy or radiolabeled in conjunction with stem cell rescue [409] show considerable promise, and such approaches are worthy of further exploration. Monoclonal antibodies may also prove to have an important therapeutic role in posttransplant B-cell lymphomas.[410,411]

A potential therapeutic target in BL worthy of special mention is EBV, because targeting viral proteins gives rise to a high degree of specificity (the few normal cells bearing EBV are likely to be eliminated with impunity). Indeed, if the property of the virus to induce cell lysis when the replication cycle is initiated could be taken advantage of therapeutically, the virus could actually be made to induce cell death. This has been shown to be possible *in vitro*.[412] Another potential target is the modified *myc* gene itself. Some years ago, proof of principle was established by demonstrating that antisense molecules could be directed toward RNA sequences present only in cells with a particular type of 8;14 chromosomal translocation, with resultant cell death. [413]

Doubtless other highly specific tumor targets will be identified in the future, and the potential of relatively nontoxic, highly specific therapy more than justifies the efforts expended on understanding the pathogenesis of the childhood NHLs. It may be some time before cancer will be treated as easily and with no more toxicity than bacterial diseases, but new approaches to the synthesis of drugs directed toward highly specific targets have brought this possibility into the realm of the thinkable. The demonstration, for example, of inhibitory effects of peptides on specific molecular targets *in vitro* can be followed by the development of peptomimetic drugs. Combinatorial chemistry allows modification of a standard chemical scaffold into millions of agents from among which structures with the desired effects can be selected—much of the process being done by computer modeling. Both approaches could lead to inhibition of genes responsible for drug resistance or nullification of the genetic defects that lead to malignancy. These advances make it highly likely that during the course of this century it will become possible to cure close to 100% of children with reduced toxicity—eventually, perhaps, if highly targeted therapy can be developed, with no more toxicity than present-day antibiotics. There remains a long way to go, however, in identifying molecular mechanisms of drug resistance and pathogenesis, and in the development of highly specific agents, although new technology and high-throughput assays are likely to greatly speed up this process. Thus, despite the exciting advances made in the treatment of childhood NHL in recent years, there is considerable room for refinement of therapy and for the introduction of newer, more highly selective approaches to treatment. This will necessitate the continued conduct of carefully designed clinical trials—the strategy that has led to the major advances made with conventional chemotherapeutic approaches.

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# 25

# LYMPHOPROLIFERATIVE DISORDERS AND MALIGNANCIES RELATED TO IMMUNODEFICIENCIES

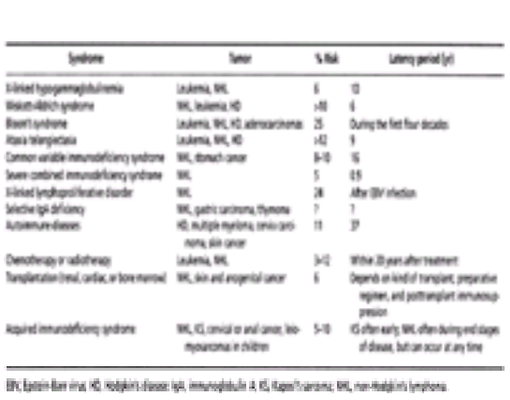
BRIGITTA U. MUELLER



## INTRODUCTION

The number of children and adults surviving despite a disturbance in their immune system is growing. Due to the success of bone marrow and solid organ transplants, and the improved prevention and treatment of infections, many children with congenital, iatrogenic, or virus-induced immunodeficiencies survive into adolescence or even adulthood. However, a prolonged immunodeficiency that is congenital, acquired, or iatrogenically induced increases the risk for the development of cancers in these children.

Estimates of the cancer incidence in patients with immunodeficiency diseases vary widely. The severity and duration of the immune defect, as well as the concurrent or preexisting infection with certain viruses, especially Epstein-Barr virus (EBV), greatly influences the likelihood of developing a malignant process. An incidence of up to 25% has been reported for patients with Wiskott-Aldrich syndrome (WAS), ataxia telangiectasia (AT), and common variable immunodeficiency (CVID) as well as for patients infected with the human immunodeficiency virus (HIV). A lower incidence has been observed after solid organ or bone marrow transplantation and patients with autoimmune disorders (Table 25-1).[1,2,3,4,5,6,7,8,9,10,11,12 and 13]

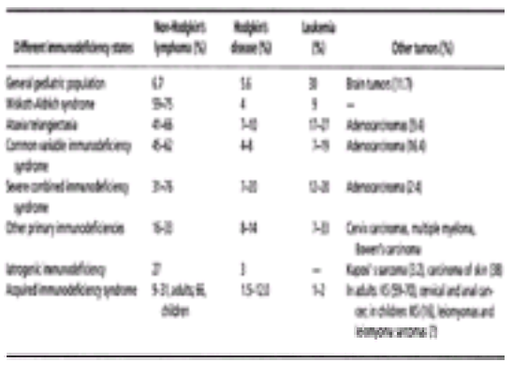


| Syndrome | Tumor | % Risk | Latency period (yr) |
|---|---|---|---|
| X-linked hypogammaglobulinemia | Leukemia, NHL | 6 | 10 |
| Wiskott-Aldrich syndrome | NHL, leukemia, HD | >10 | 6 |
| Bloom's syndrome | Leukemia, NHL, HD, adenocarcinoma | 25 | During the first four decades |
| Ataxia telangiectasia | Leukemia, NHL, HD | >32 | 9 |
| Common variable immunodeficiency syndrome | NHL, stomach cancer | 8–10 | 16 |
| Severe combined immunodeficiency syndrome | NHL | 5 | 0.5 |
| X-linked lymphoproliferative disorder | NHL | 24 | After EBV infection |
| Selective IgA deficiency | NHL, gastric carcinoma, thymoma | ? | ? |
| Autoimmune diseases | HD, multiple myeloma, cervix carcinoma, skin cancer | 13 | 27 |
| Chemotherapy or radiotherapy | Leukemia, NHL | 3–12 | Within 20 years after treatment |
| Transplantation (renal, cardiac, or bone marrow) | NHL, skin and anogenital cancer | 6 | Depends on kind of transplant, preparative regimen, and post-transplant immunosuppression |
| Acquired immunodeficiency syndrome | NHL, KS, cervical or anal cancer, leiomyosarcoma in children | 5–10 | KS often early, NHL often during end stages of disease, but can occur at any time |

EBV, Epstein-Barr virus; HD, Hodgkin's disease; IgA, immunoglobulin A; KS, Kaposi's sarcoma; NHL, non-Hodgkin's lymphoma

**TABLE 25-1. INCIDENCE OF MALIGNANCIES IN IMMUNE DEFICIENCY DISORDERS**

Lymphoproliferative disorders (LPDs), or, in the case of transplant patients, posttransplantation lymphoproliferative disorders (PTLDs), predominantly of B-cell origin, are the most common neoplasias in patients with congenital or acquired immunodeficiencies (Table 25-2).[8] They represent a heterogeneous group of diseases that can range from reactive polyclonal hyperplasia, which, in the presence of severe immunosuppression can be fatal, to true monoclonal malignant lymphomas. There is often no clear distinction made between non-Hodgkin's lymphoma (NHL) or Hodgkin's disease (HD), which need to be treated with chemotherapy, and the polyclonal or oligoclonal lymphoid proliferations, which tend to respond to a decrease in immunosuppression. Although this chapter tries to distinguish between the two presentations where deemed appropriate and necessary, they probably represent a spectrum of the disease, and considerable overlap is to be expected.



| Different immunodeficiency states | Non-Hodgkin's lymphoma (%) | Hodgkin's disease (%) | Leukemia (%) | Other tumors (%) |
|---|---|---|---|---|
| General pediatric population | 4.7 | 3.6 | 38 | Brain tumors (11.7) |
| Wiskott-Aldrich syndrome | 59–75 | 4 | 9 | — |
| Ataxia telangiectasia | 41–66 | 1–10 | 11–27 | Adenocarcinoma (3.4) |
| Common variable immunodeficiency syndrome | 8–42 | 4.4 | 1–19 | Adenocarcinoma (16.4) |
| Severe combined immunodeficiency syndrome | 31–78 | 1–10 | 12–28 | Adenocarcinoma (2.4) |
| Other primary immunodeficiencies | 16–33 | 8–14 | 1–13 | Cervix carcinoma, multiple myeloma, Bowen's carcinoma |
| Iatrogenic immunodeficiency | 27 | 3 | — | Kaposi's sarcoma (3.2), carcinoma of skin (38) |
| Acquired immunodeficiency syndrome | 9–31 adults; 66, children | 1.5–12.3 | 1–2 | In adults: KS (59–78), cervical and anal cancer; in children: KS (16), leiomyoma and leiomyosarcomas (7) |

**TABLE 25-2. DISTRIBUTION OF NEOPLASIAS ASSOCIATED WITH DIFFERENT IMMUNODEFICIENCY STATES**

Also clearly increased is the incidence of Kaposi's sarcoma (KS) (especially in HIV disease and after solid organ transplantation), adenocarcinomas (in patients with CVID or AT), and leiomyosarcoma (in children with HIV infection or after solid organ transplants). Furthermore, bone marrow transplant recipients remain at an increased risk for the development of secondary cancers, even after the immunosuppression has been reversed (see Chapter 49).

Several registries and collaborative groups have begun to focus on malignancies in immunocompromised hosts. The immunodeficiency cancer registry collects data

on neoplasms occurring in patients with primary immunodeficiencies.[14] The national and international bone marrow and solid organ transplant registries keep track of the development of cancers in transplanted patients (e.g., Cincinnati Transplant Tumor Registry), and the National Cancer Institute supports the AIDS (acquired immunodeficiency syndrome) Malignancy Consortium and its AIDS malignancy tissue bank.[13,15,16] and [17]

## EPIDEMIOLOGY AND CLINICAL PRESENTATION

### Inherited Immunodeficiency Syndromes

Primary, congenital immunodeficiency disorders include a heterogeneous group of syndromes, which can become clinically manifest as early as at birth, but also not until adulthood. Abnormalities in the humoral immune system are due to a defective development of B cells, whereas disturbances in the maturation of T cells lead to defects in cellular immunity.[18] Morbidity and mortality are mainly due to infections or to a malignancy. [8] Because primary immunodeficiency syndromes are rare, and each treatment center will only diagnose a few cases of associated cancers, an international registry of these cases was established in 1973 supported by the National Cancer Institute.[14]

It has been estimated that patients with inherited immunodeficiencies are almost 10,000 times more likely to develop cancer, especially neoplasia of the lymphoreticular system.[19] Between 1% and 4% of patients with inherited immunodeficiencies develop lymphomas. A recent review of the Immunodeficiency Cancer Registry in Minneapolis, Minnesota, revealed that NHL constituted 50.4% of the reported tumors, followed by leukemia (12.6%), adenocarcinoma (9.2%), and HD (8.6%). Almost 20% of the patients had “other” tumors.[8] A prevalence of NHL over the leukemias (65% versus 8%), a distribution that is markedly different from the general pediatric population, is observed in children with primary immunodeficiencies. [20]

#### *Wiskott-Aldrich Syndrome*

WAS is an X-linked recessive disorder characterized by immunodeficiency, profound thrombocytopenia with small platelets, and eczema. [21] In the past, most of these boys died during the first decade of life from hemorrhage or infection, but recently life expectancy has increased due to better supportive care. [18] Both the humoral and cellular immune system are affected, reflected by low serum immunoglobulin M (IgM) levels, elevated IgA and IgE levels, and a progressive loss of T cells combined with an expansion of the B-cell pool. The gene that is defective in WAS has been identified and maps to Xp11.23. [22] WAS is caused by mutations in an intracellular protein (WASP) that is involved in signal transduction and regulation of the rearrangement of the actin cytoskeleton.

The risk of developing a malignancy approaches 100% by 30 years of age in patients with WAS. [2] Up to 75% of the cancers in WAS are NHL, and 50% of patients have widely disseminated disease at diagnosis.[2] In a multi-institutional survey of 154 pediatric patients with WAS, Sullivan, et al. [1] documented 21 patients (13%) with a malignancy. The average age of onset was 9.5 years, and the majority of the malignancies involved the lymphoreticular system. Burkitt's lymphoma (BL), and lymphoblastic lymphomas are the predominant tumor types, but in contrast to NHL in patients with a normal immune system, these tumors occur more frequently in extranodular sites. HD, mostly of the nodular sclerosing type, is approximately ten times less frequent than NHL. Other malignancies, including myelogenous leukemia, cerebellar astrocytoma, and smooth muscle tumors, have also been described in patients with WAS.

The etiology of the malignancies in WAS remains unclear, but viral stimulation, such as with EBV, and defective intracellular signaling have been postulated. [22,23] Recent data also suggest a pivotal role for WASP in normal hematopoiesis, but it is not yet clear whether a disturbance will increase the risk for the accumulation of potential oncogenic mutations.[24]

#### *X-linked Lymphoproliferative Disorder*

X-linked LPD (XLP) or Duncan's syndrome is characterized by a striking predisposition to fatal EBV infections. [25] A mutation at Xq25 impairs the ability of males to mount an effective immune response to EBV. As of 1995, more than 270 affected males within 80 unrelated families have been reported to the XLP disease registry. [26] Fulminant infectious mononucleosis (IM) with a typical virus-associated hemophagocytic syndrome was seen in 80% of the boys (median age of onset, 5 years). This is a highly fatal disease, with only 5 per 132 (4%) surviving. Eighty-two (30%) of the boys developed an LPD with a median age of onset of 6 years, and only one-third of patients survived this manifestation.[26] A small subgroup of patients develop vasculitis and pulmonary lymphomatoid granulomatosis, which appears to be a T-cell process (see below).[27]

The gene defect responsible for XLP has been cloned. The SH2-domain–containing gene 1A (SH2D1A) encodes a small protein of 128 amino acids containing a single SH2 domain, which is thought to play an important role in signal transduction in activated T cells. [28]

#### *Hyper-Immunoglobulin M and E Syndromes*

X-linked hyper-IgM syndrome is a heterogeneous immunodeficiency disorder characterized by decreased serum IgG and IgA levels, whereas IgM levels are markedly elevated. Clinically, it is manifested by frequent respiratory infections, diarrhea, and liver infections with parasitic and opportunistic organisms. [29] Benign and malignant lymphadenopathy is observed in a substantial subgroup of patients, and several patients have been described who developed tumors of the pancreas, liver, and biliary tree.[8,29,30] The disease is caused by a mutation in the gene encoding the CD40 ligand, an integral transmembrane glycoprotein, which is expressed mainly in CD4-positive cells.[31,32] This protein plays a crucial role in B-cell activation and isotype switching. [33]

The hyper-IgE syndrome, a disease characterized by eczematous skin rash, recurrent staphylococcal abscesses, and purulent otitis media, has also been associated with an increased incidence of lymphoid tumors.[8,34,35] These patients appear to have an underlying dysfunction of the interleukin 12 (IL-12)/interferon-gamma (IFN-g) pathway.[36]

#### *Selective Immunoglobulin A Deficiency*

Unlike most immunodeficiency disorders, selective IgA deficiency is not associated with an increase in lymphoid but rather with an excess of epithelial malignancies.[8,19,37,38] The Immunodeficiency Cancer Registry lists 38 patients with IgA deficiency and cancer, but only six (15.8%) had NHL and three (7.9%) had HD, whereas the predominant tumors were adenocarcinomas, including gastrointestinal carcinomas.[8] This is probably an inaccurate estimate of the true incidence of cancers, as IgA deficiency occurs in approximately 1 in 600 people but is rarely diagnosed. [21]

#### *Severe Combined Immunodeficiency and Common Variable Immunodeficiency*

Severe combined immunodeficiency (SCID) and CVID include a broad range of immunodeficiency diseases affecting both the humoral and cellular systems. NHLs account for 75% of the malignancies in patients with SCID; HD accounts for an additional 10%. [8] Median age at diagnosis for NHL is 1.6 years and 0.3 years for HD, contributing to the high mortality rate in early childhood of this disease.

CVID is the most common immunodeficiency syndrome and has a broad spectrum of clinical manifestations, including late-onset hypogammaglobulinemia, recurrent sinopulmonary infections, and autoimmune disorders. The incidence of cancers has been estimated to be 2.5% in patients with onset of the disease before the age of 16 years, with an increase to 8.5% in patients with later onset. [39] Forty-six percent of the malignancies in CVID are NHL, 7% each are HD and leukemias, and approximately 17% are adenocarcinomas, but a variety of other tumors has also been reported. [10,40,41]

Cartilage-hair hypoplasia, an autosomal-recessive metaphyseal chondrodysplasia, is one of the most common immunodeficiency syndromes in Finland, occurring in 1 in 23,000 live births.[42] Patients not only exhibit lymphopenia and decreased *in vitro* lymphocyte reactivity, but have recently also been found to have an increased incidence of cancers, mainly of the NHL type, and of basal cell carcinomas. [43]

#### *Chediak-Higashi Syndrome*

Chediak-Higashi syndrome is a functional disorder of granulocytes, with autosomal recessive inheritance. [21] It is the only primary phagocyte defect that has been associated with an increased risk for the development of neoplasms. Partial oculocutaneous albinism, photophobia, and increased susceptibility to pyogenic infections characterize the disorder. Laboratory evaluations show abnormally large lysosomal inclusions in monocytes, neutrophils, and lymphocytes, as well as impaired chemotaxis and bactericidal activity and abnormal natural killer cell function. [44] The majority of patients enters an accelerated and usually fatal phase during the first decade of life manifested by pancytopenia, fever, jaundice, hepatosplenomegaly, lymphadenopathy, and neurologic changes. Histologically, a diffuse mononuclear cell infiltration, reminiscent of a virus-associated hemophagocytic syndrome, is observed. [8]

### *Bloom's Syndrome*

Bloom's syndrome is an autosomal-recessive disorder characterized by short stature, pigmentary changes (e.g., café-au-lait spots), immunodeficiency leading to repeated infections during infancy and childhood, chronic lung disease, and a strong predisposition to cancer. Chromosomal instability, typically with an elevated level of sister chromatid exchanges, is seen on cytogenetic examinations. Bloom's syndrome (similar to Fanconi's anemia) is a clastogenic disorder.

As of 1996, 100 cases of cancer occurring in 71 of 168 patients followed through the Bloom's Syndrome Registry had been observed. [45] The predominant neoplasms are skin cancers (with unusual locations) and leukemias, but a variety of other malignancies are also observed. [46] The age of onset for carcinomas is exceptionally early.

### *Ataxia Telangiectasia*

AT, inherited as an autosomal-recessive disorder with an incidence of approximately 1 in 300,000, presents in early childhood with progressive cerebellar ataxia, oculocutaneous telangiectasia, radiosensitivity, and immunodeficiency, with defects in both cellular and humoral immunity. [47] Patients with AT represent approximately 30% of all cases of cancers reported to the Immunodeficiency Cancer Registry. [8] A review of 263 patients noted a 252-fold excess of lymphomas in white patients with AT, whereas black patients had a 750-fold higher incidence than the normal population. [3]

An estimated 12% to 40% of patients with AT develop a malignancy.[4,48] Children with AT have a predisposition to B-cell malignancies, whereas T-cell malignancies are prominent in adults.[5,6] Overall, there is a predominance of T-cell malignancies over B-cell lymphomas (6:1), which is different from the general population. [5] HD tends to occur in a younger age group (mean age, 7.8 years versus 11.5 years), and a large proportion of patients have HD of the mixed cellularity (42%) or lymphocyte depleted type (33%), whereas the nodular-sclerosing type is most commonly seen in the general pediatric population. [49,50] However, 31 solid tumors (e.g., brain tumors, gastric carcinomas, ovarian, and uterine tumors) were noted among 119 patients with AT and a malignancy. [4] Heterozygotes (approximately 3% of the population) may also be at increased risk for cancer, as demonstrated in patients with breast cancer. [51]

At least four complementation groups have been identified, and all map to the ATM gene on chromosome 11q22-q23. [52] AT cells are abnormally sensitive to killing by ionizing radiation, and abnormally resistant to inhibition of DNA synthesis by ionizing radiation. Recent evidence suggests the presence of a tumor-suppressor gene localized in 11q22-q23, important at least for the development of B-cell chronic lymphocytic leukemia. [53] Furthermore, the 11q22-q23 deletions appear to define a subset of B-cell chronic lymphocytic leukemia characterized by extensive lymph node involvement, rapid disease progression, and poor survival. [54]

## Iatrogenic Immunodeficiency States

### *Transplant Patients*

In 1997, the Cincinnati Transplant Tumor Registry contained data on 512 pediatric patients who developed 527 tumors, and 9,639 adults who developed 10,286 neoplasms after solid organ transplantation. [55] The most common tumor (52%) in pediatric transplant recipients was PTLDs, and these were relatively more common in nonrenal transplant patients compared to renal patients (81% to 31%). [55]

Skin cancer, an extremely rare occurrence in the general pediatric population, comprised 19% of all tumors (31% in renal versus 6% in nonrenal patients), but the majority presented 10 years after transplant, beyond the pediatric age group. [55] Other tumors included sarcomas (with a preponderance of leiomyosarcomas and KSs), carcinomas of the vulva, cervix and perineum, and liver or thyroid. A minority of patients developed the more “typical” malignancies of childhood, leukemias or brain tumors (the latter only in bone marrow transplant patients). The majority of tumors became manifest during childhood (before 18 years of age), and 40% were diagnosed in former pediatric transplant recipients when they were between 19 and 40 years old. [55]

Although it is clear that EBV infection plays a major role in transplant-associated LPDs (see following sections), these patients also experience an excessive number of EBV-negative NHLs.[56,57] These EBV-negative tumors tend to occur mainly in adults, and there is no correlation with the time elapsed since transplant, although they are more common a year or more after transplant.[56,57 and 58]

### Renal Transplants

Posttransplant LPDs develop in approximately 1% of renal allograft recipients and are usually manifested during the first few years after transplant, involving the allograft in up to 18%.[13,55,59,60] In a group of 81 children followed at Children's Hospital of Pittsburgh, 19 (23%) developed symptomatic EBV infection. [61] Of these, seven had IM, ten developed a PTLD, and two a malignant NHL. Transplantation of a kidney from an EBV-positive donor into an EBV-negative recipient correlated with a very high probability for seroconversion and for the development of a PTLD or malignancy ( $p$ <.001).[61] Although some studies have indicated no association with the immunosuppression regimen,[62] others have found an increased risk for the development of malignancies in patients who received tacrolimus posttransplant.[61,63]

### Cardiac and Pulmonary Transplants

Almost 4% of heart transplant patients develop a malignancy during their first year after transplant (32% lymphoid, 34% skin, and 27% other). More than 8% developed a malignancy within the first 4 years, mainly nonlymphoid tumors (12% lymphatic, 54% skin, 30% other). [16] In fact, the nonlymphoid tumors represent the second most common cause of death 4 and 5 years posttransplant (18.6%), whereas lymphoid malignancies (NHL and PTLD) account for only 3% to 4% of the deaths 1 year or later after transplant.[16] Pediatric heart transplant patients have a lower risk of malignancies (1.4% after 1 year and 2.0% after 3 years of follow-up). [15,56]

A similar distribution is also observed after lung transplants, although the overall incidence of malignancies is lower (4.8% within the first year and 4.3% within 4 years of transplantation). Lymphoid malignancies predominate during the first year (56%), whereas skin cancers become the most common neoplasm (56%) 4 years after lung transplantation.[16] Children had a slightly higher incidence of malignancies within the first year (7.6%) but a similar rate after 3 years of follow-up (2.1%). [15]

The clinical presentation of tumors after cardiac or lung transplants depends on the localization of the tumor and can be associated with lymphadenopathy and splenomegaly, gastrointestinal symptoms (sometimes even perforation of gut), nasopharyngeal obstruction, respiratory distress with pulmonary infiltrates, fever of unknown origin, anemia or neutropenia, skin lesions, or neurologic symptoms. [64,65]

### Gastrointestinal Transplants

The most common malignancy after gastrointestinal transplants is PTLD, followed by nonmelanoma skin cancers. [66] In 1999, the Intestinal Transplant Registry listed an 8% incidence of LPDs in patients after intestinal transplantation, and a 14% and 15% incidence, respectively, after liver and intestine or multivisceral transplant. NHLs were the cause of death in 14% of patients. [17] Among children undergoing intestinal transplantation, the incidence for PTLD is between 8% and 20%. [67,68 and 69] The main locations for PTLDs are the allograft (liver), abdominal or peripheral lymph nodes, and the lungs. [67,68,70] An increase in gammaglobulin levels and the appearance of mono- or oligoclonal Ig production or hypoalbuminemia have been recognized as early warning symptoms. [68,70]

### Bone Marrow Transplants

In addition to immunosuppression, a number of factors contribute to the risk of malignancy after bone marrow transplantation. Among almost 20,000 bone marrow transplant recipients, a marked increase in risk was related to age at the time of transplant. Children younger than the age of 10 years had a 36.6 times higher risk, whereas adolescents and young adults between 10 and 29 years old had a 4.6 times higher risk, with a close to expected risk in patients transplanted at older than 30 years of age.[71] The same investigators evaluated the risk for PTLDs after bone marrow transplantation and documented 78 cases among 18,014 patients (0.4%). [71] The majority occurred during the first year after transplant; in fact, the highest incidence was seen during the first 5 months (120 cases per 10,000 patients). [71]

Whereas PTLDs are much more common in patients after allotransplantation, solid tumors occur both after allogeneic and autologous transplants. [72] Total body irradiation clearly plays an important role, but at least one study also found a correlation with the use of antithymocyte globulin and the risk of malignancy. [73,74] Not surprisingly, patients with Fanconi anemia have the highest incidence of malignant tumors after transplant. [72] Patients with aplastic anemia have been shown to have a comparable risk for secondary cancers with immunosuppression alone [relative risk (RR) = 5.15] and bone marrow transplant (RR = 6.67) compared with a normal population; however, the cumulative risk after 10 years was markedly higher after immunosuppression alone (18.8% versus 3.1%). [75]

Twenty-five solid tumors and 20 cases of PTLDs were documented among 3,182 children who received an allogeneic bone marrow transplant. [76] Risk factors for PTLDs included chronic graft-versus-host disease (GVHD) (RR = 6.5), unrelated or HLA-mismatched donor (RR = 7.5), T-cell depleted graft (RR = 4.8), and antithymocyte globulin therapy (RR = 3.1).[76] The PTLDs occurred a median time of 1.5 years after transplantation, and all patients with PTLDs died. Solid tumors occurred most frequently in patients who had received high-dose total body irradiation (RR = 3.1) and in children transplanted at a younger age (RR = 3.7). The median time from transplantation was markedly longer (6 years; range, 0.3 to 14.0 years), and the predominant tumors were brain and thyroid cancers. [76] The excess of these tumors is probably at least in part a consequence of irradiation (see Chapter 13), but the four cases of melanoma and three cases of squamous cell carcinoma of the tongue may have been due to the immunosuppression.

#### *Chemotherapy-Induced Immunosuppression*

Second malignancies due to certain chemotherapeutic agents and radiation are discussed in Chapter 49 and are thought to be the result of DNA damage or the acquisition of mutations. The relationship between the chemotherapy-induced immunosuppression and de novo malignancies has been less well studied, although it is well known that chemotherapy leads to a prolonged state of lymphopenia, especially affecting the T-cell numbers. [77] Malignancies (especially NHL) have been described to occur at a higher incidence in patients treated with either cyclophosphamide or azathioprine for rheumatoid arthritis. [78,79,80 and 81]

### Autoimmune Disorders

Autoimmune disorders have been associated with an increased incidence of malignancies, but it is not entirely clear whether this is due to the underlying disease or the treatment for it (e.g., immunosuppressive chemotherapy). Patients with lupus erythematosus have been described to develop lymphoid malignancies (e.g., Castleman's disease and reticulum-cell sarcoma), and women have a slightly increased risk to develop carcinomas of the cervix, lung, and breast. [82,83 and 84] Patients with systemic sclerosis had an increased risk for lung cancer [standardized incidence ratio (SIR), 4.9], nonmelanoma skin cancer (SIR, 4.2), and primary liver cancer (SIR, 3.3).[85] The incidence of hematologic cancers, especially NHL, is elevated in adult patients with Sjögren's syndrome. [86] Rearrangements of both the heavy chain and light chain Ig genes were found in the salivary glands of these patients. [87]

### Acquired, Virally Induced Immunodeficiencies

#### *Human Immunodeficiency Virus Type 1*

The Joint United Nations Programme on AIDS (UNAIDS) estimates that more than 5.4 million new infections with HIV-1 occur every year (including 620,000 children), and that currently 1.4 million children younger than the age of 15 years and 34.7 million adults are living with HIV/AIDS (UNAIDS; http://www.unaids.org/). As of June 2000, more than 8,800 children younger than 13 years had been diagnosed with AIDS in the United States. [88]

Each year, approximately 130 cases of cancer are diagnosed per million non–HIV-infected children (0.013%). The classification of pediatric AIDS includes (as in adults) primary brain lymphomas, small noncleaved cell (BL) NHLs, immunoblastic or large cell lymphoma of B-cell or unknown immunologic phenotype, as well as KS, as AIDS-defining events (category C), whereas leiomyosarcomas are included in category B as a sign of a moderately symptomatic stage. [89]

AIDS-defining malignancies have been reported to the Centers for Disease Control and Prevention in approximately 2% of all HIV-infected children, compared to 10% to 15% of adults. The cumulative statistics in children list BL and immunoblastic NHL in approximately one-third of cases each, followed by primary lymphoma of the central nervous system (CNS) (19%) and KS (17%).[90] However, because only the initial AIDS-defining event is reported, this probably represents an underestimate of cases of cancer in children with more advanced disease. A survey conducted by the Children's Cancer Group and the National Cancer Institute identified 57 HIV-infected children diagnosed with cancers between July 1982 and January 1997. Twenty-five (44%) of these children would not have been captured through the current Centers for Disease Control and Prevention classification system. [91] In a study of men with, or at risk for, HIV infection conducted in southern Europe, a total of 19,609 person-years of observation among HIV-positive men were compared with 7,957 person-years among HIV-negative men. A statistically significant increase for HD (SIR = 8.7) and liver cancer (SIR = 11) was observed in the HIV-positive cohort. [92] Neither disease is currently included in the list of AIDS-defining malignancies.

The incidence of HIV-associated tumors appears to be different in developing countries. An increasing number of KSs have been reported to occur in both children and adults in Africa, whereas the number is decreasing in the United States and Europe, most likely due to the introduction of highly active antiretroviral therapy, including protease inhibitors. [12,93,94,95,96 and 97] Furthermore, a trend to an increase in retinoblastomas, nasopharyngeal carcinomas, and rhabdomyosarcomas, pediatric tumors not commonly associated with immune deficiency states, has been described.[93,94] Seventy-six consecutive newly diagnosed malignancies were evaluated for HIV infection in a study from Zimbabwe.[98] Twenty-seven of 64 children were HIV seropositive (seroprevalence = 42.2%), and the most common malignancies were NHL (22.4%), acute lymphoblastic leukemia (19.7%), Wilms' tumor (19.7%), and KS (15.8%). Nine of 17 patients with NHL and all 12 patients with KS were HIV positive.

### Kaposi's Sarcoma

In the United States, KS is most prevalent in homosexual or bisexual HIV-infected men (95% of adult KS cases) and is rare in men or women infected through heterosexual contacts, intravenous drug use, or transfusions.[99] It has been estimated that the risk for developing KS is 13,000 times higher for an HIV-infected person.[11,12,100,101] The proportion of patients with KS as an AIDS-defining diagnosis has decreased in the United States and Europe from more than 30% of newly diagnosed AIDS cases in the early 1980s to less than 5% in the late 1990s since the advent of highly active antiretroviral therapy. [90] However, a continued increase in the incidence of KS is observed in both sexes in Africa, and KS is now the second most common tumor in African women.[95]

KS is the AIDS-defining illness in less than 1% of children younger than 13 years of age in the United States, and only 3% of adolescents between 13 and 19 years of age, but the incidence increases to 9% in young adults between 20 and 24 years of age, and to 13% in adults older than 25 years of age. [90,102] However, a significant increase in the incidence of KS has been reported to occur in children from African countries. [93,94,103] In Zambia, KS comprised 6% of all childhood cancer cases before 1986 and increased to 19.3% between 1987 and 1992. Furthermore, the male to female ratio has changed from 3:1 before the AIDS epidemic to approximately 1.7:1.0.[94] In another study from Zimbabwe, KS comprised 10.3% of all childhood tumors.[95]

The age of onset of KS in childhood is variable. The median time of onset in vertically infected children is 33 months (range, 2 to 98 months), with a peak incidence in young children (mean, 5.6 years; range, 7 months to 14 years).[93,94] In contrast to adults, KS is rarely the first presentation of HIV disease in children, but follows symptoms of failure to thrive, recurrent infections, and hepatosplenomegaly. [93]

### Non-Hodgkin's Lymphoma and Hodgkin's Disease

The relative risk of developing NHL has been estimated to be 200 to 300 times higher in HIV-infected patients. [104] In a study of 1,255 patients (906 males and 349 females) followed by the HIV Italian Seroconversion Study Group, a 157-fold increase in NHL was observed. [105] The absolute risk for brain NHL is even higher, and has been estimated to be 3,600-fold (incidence of 0.5% to 1.0%). [106] Only approximately 1% of children with AIDS younger than the age of 4 years will ever have NHL, but this number increases steadily with age, and almost 6% of AIDS patients older than 60 years will develop an NHL.

In one study of HIV-infected homosexual men, an excess incidence of 19.3 cases of HD per 100,000 person-years was documented. [107] A similar result was published by Lyter et al.[108] in patients followed as part of the national Multicenter AIDS Cohort Study. The ratio of NHL to HD appears to be influenced by the route of acquisition of HIV disease, with a ratio of 2:1 for intravenous drug users and a ratio of 12.4:1.0 in homosexual men. [107,109,110] HD is relatively rare in children younger than the age of 15 years, although several cases have been described in young HIV-infected children. [91,110,111 and 112]

### Smooth Muscle Tumors

Smooth muscle tumors (leiomyomas and leiomyosarcomas) are more common in HIV-infected children compared to adults.[91,112,113,114,115,116,117 and 118] These tumors are rare in otherwise healthy children, accounting for less than 2% of all childhood cancers, and are much less common than other soft tissue tumors (e.g., rhabdomyosarcomas). The annual incidence is estimated to be eight cases per million children. However, an increased number of leiomyomas and leiomyosarcomas has been described in HIV-infected children, [91,113,114 and 115,119,120] and this tumor is now included as a category B symptom in the revised 1994 classification of pediatric HIV disease.[89] In our survey of the cases reported to the Children's Cancer Study group and the National Cancer Institute, 17% of all reported tumors (eight) were either leiomyomas or leiomyosarcomas, and we are aware of at least 20 cases that have occurred in HIV-infected children in the United States. [91,114] To date, no similar increase in incidence has been observed in adults, but several case reports of smooth muscle tumors occurring in unusual locations (e.g., brain, spleen, and lungs) have been reported. [120,121]

### Cervical and Anal Neoplasms

An increased incidence of cervical pathologies has been noted in HIV-infected women, and the revised classification for HIV-infected adults from 1993 includes cervical dysplasia (moderate or severe) as well as cervical carcinoma *in situ* as an AIDS-defining diagnosis in HIV-infected women and adolescents.[122,123,124,125,126,127 and 128] The prevalence of cervical dysplasia has increased in all adolescents, and human papillomavirus (HPV) infection is found in 15% to 38% of sexually active adolescents and in up to 77% of HIV-infected teenagers. [129,130] In one study, more than 30% of 133 HIV-infected girls (age 13 to 18 years) had squamous intraepithelial lesions, three times more than in an HIV-negative control group. [130] An invasive cervical carcinoma has been described in a 16-year-old girl with HIV infection.[125] At the same time, the incidence of anal cancers appears to be increased in homosexual males with HIV infection. [131,132]

### Other Tumors

Although not part of the AIDS definition, it is notable that other tumors occur with an increased frequency as well. Studies from Zimbabwe and Uganda not only reported a marked increase in the incidence of KS and NHL but also of squamous cell tumors of the conjunctiva. [95,96] Whether this is a true manifestation of the AIDS epidemic or a phenomenon associated with better surveillance and reporting remains to be determined.

#### *Human Immunodeficiency Virus Type 2*

HIV-2 is only superficially related to HIV-1 and is most prevalent in West and Southern Africa, with a seroprevalence in the population of up to 8%. [133,134,135,136 and 137] Modes of transmission are the same as for HIV-1, but the onset and progression of immunodeficiency and associated diseases is markedly slower. Although patients often are co-infected with human herpesvirus 8 (see section on Human Herpesvirus 8 or Kaposi's Sarcoma–Associated Herpesvirus), development of KS occurs at a lower frequency than in HIV-1 infected patients.[138] However, women are still at an increased risk to develop cervical neoplasia. [139]

#### *Human T-Cell Lymphotropic Virus Type I*

Infection with human T-cell lymphotropic virus type I (HTLV-I) is endemic in the Caribbean, Central and South America, certain areas of Africa, and Japan. [140] The virus is transmitted sexually, from mother to child, through transfusion of infected blood products, and by sharing used needles. [140,141 and 142] More than 98% of all infected persons remain asymptomatic, but HTLV-I myelopathy or adult T-cell leukemia can develop in a minority of patients. [140,141] Adult T-cell leukemia usually occurs after 20 to 30 years of infection, and it is therefore not surprising that only a few case reports in children and adolescents have been published. [143,144]

## PATHOGENESIS

The understanding of the pathogenesis of tumors in immunocompromised patients is still evolving. A single event rarely leads to the emergence of a malignancy, but when a degradation of host defense mechanisms permits infection with ubiquitous viruses, the potential for malignancy increases. Indeed, insights gained in studying immunodeficiency disorders has provided an appreciation of the immune surveillance system and may lead to new therapeutic approaches.

### Genomic Instability

Chromosomal translocations occur in the majority of NHLs and in many leukemias. This is probably a product of the genetic variability of lymphocytes to create and maintain antigen receptor diversity.[145] Patients with AT have an increased susceptibility to chromosome translocations, but only a subgroup of these result in the activation of an oncogene.[48,146,147] Cells from patients with Bloom's syndrome show a chromosomal instability that leads to a number of spontaneous chromosomal abnormalities.[45,145] Almost 100% of mice with both the *Scid* and p53-null mutation (*Scid* p53–/–) develop disseminated lymphomas, and they have been found to have a chromosomal translocation involving the IgH locus near the telomere of chromosome 12.[148,149] The loss of p53 control allows unrestricted growth of this abnormal clone. The continued stimulation by certain viruses (e.g., HIV and EBV) increases the stochastic possibility that such an event will occur.

The etiology of malignancies in WAS remains unclear, but viral stimulation (e.g., EBV) and defective intracellular signaling have been postulated. [22,23] Recent data also suggest a pivotal role for WASP in normal hematopoiesis, but it is not yet clear whether a disturbance increases the risk for the accumulation of potential oncogenic mutations.[24] Patients with XLP mount an uncontrolled polyclonal proliferation of T and B cells after exposure to EBV. It is currently thought that a defect in cytotoxic T cells leads to uncontrolled B-cell proliferation. [150,151]

### Immunosuppression and Iatrogenic Factors

One major risk factor for the development of a malignancy is clearly the degree of immunosuppression. In a review of 89 pediatric liver transplant recipients who received tacrolimus for immunosuppression, there was a 20% incidence of PTLDs, compared to 3% in a historical control group who received cyclosporin. [68] Among 298 children undergoing liver transplantation, the incidence for PTLD was 8.4%. [67] The risk of developing a PTLD went up to 28% when both OKT3 and tacrolimus were used simultaneously. In this series, children who developed a PTLD had a mortality rate of 60%. [67] Bone marrow transplant recipients who received an HLA-mismatched or T-cell–depleted allograft, or patients who were treated with antithymocyte globulin or anti-CD3 antibody for prophylaxis of GVHD, are at highest risk for the development of PTLD.[71] Late-onset PTLD, on the other hand, is most closely related to continued immunosuppression for the treatment of chronic GVHD. In summary, commonly recognized risk factors for the development of a PTLD include donor-recipient mismatch or severe GVHD (both necessitating more aggressive immunosuppression), T-cell depletion of donor marrow, or the use of anti–T-cell monoclonal antibodies or high-dose antithymocyte globulin for GVHD prophylaxis.

The risk for an HIV-infected patient to develop a cancer increases with the progressive weakening of the immune system. [152] However, the CD4 count alone is not an absolute determinant for a child's risk to develop a tumor, because HIV-infected children can develop a malignancy when their CD4 count is only moderately depressed. The duration of immunosuppression might play a role, and if so, this could have an important impact on the number of HIV-associated malignancies in the

future as children live longer with HIV infection.

It is currently recommended that all pregnant HIV-positive women receive zidovudine during pregnancy and birth, and that their infants be treated for the first 6 weeks of life to decrease the risk for transmission of HIV infection.[153] Zidovudine given during gestation is incorporated into the DNA of newborn mice and monkeys, as well as into the nuclear DNA of cord blood samples drawn from children whose mothers had been treated with the drug. An increased risk to develop liver and lung tumors, as well as tumors of the reproductive organs, has been observed in the offspring of mice that were treated with zidovudine during the last trimester.[154,155] However, to date there is no indication that children exposed to zidovudine *in utero* are at an increased risk to develop a malignancy, and the benefit of perinatal treatment with zidovudine currently outweighs the risk. Continued vigilance and follow-up observation of all children exposed *in utero* or postnatally to zidovudine is clearly necessary.

## Infection with Viruses

Virus-induced cancers account for approximately 15% to 20% of all cancers worldwide.[156] Although infection with viruses is usually well tolerated and associated with self-limited disease in otherwise healthy hosts, it can have devastating effects in the immunocompromised patient, by triggering an uncontrolled lymphoproliferative process, or, as in the case of HIV and HTLV-1 infection, by inducing an immunocompromised state.

### *Epstein-Barr Virus*

EBV is a ubiquitous human herpesvirus that primarily results in asymptomatic infections or IM during childhood and adolescence. Infected individuals become life-long carriers of the latent form of the virus. EBV has the capability to infect epithelial cells, documented by positive cultures from patients with IM, undifferentiated nasopharyngeal carcinoma, and patients with hairy leukoplakia, an AIDS-associated epithelial lesion of the oropharynx.[157,158] However, chronically infected B lymphocytes are the main reservoir, and the magnitude of this reservoir is closely related to the degree of immunodeficiency, or, more specifically, the capability of memory T cells to mount an EBV-specific cytotoxic T-cell response.[159]

EBV is capable of transforming normal B lymphocytes into continuously proliferating cell lines *in vitro*. Such cell lines produce little or no virus because virus replication results in cell death, and the viral information persists as circular DNA episomes located in the cell nucleus. Latently infected lymphocytes express at least nine viral proteins, including six EBV nuclear antigens (i.e., EBNA-1, -2, -3a, -3b, -3c, and leader protein) and three latent membrane proteins (i.e., LMP-1, -2a, and -2b), as well as two small nonpolyadenylated EBV-encoded RNAs (i.e., EBER-1 and -2). LMP-1 has potent transforming effects both in cell cultures as well as in animal models and plays an essential role in the transformation of B cells.[160,161] Both EBNA-2 and EBNA-3c (and probably also EBNA-3a) activate LMP-1 and therefore have oncogenic potential.[156,162,163] LMP-1, in turn, enhances bcl-2 expression (an anti-apoptosis gene) and activates natural killer NK-kB and c-jun, protecting EBV-infected cells from cell death.[164,165]

By providing the targets for immune recognition by EBV-specific T cells, the EBV-latent genes ensure that transformed cells will be destroyed by the normal immune system. Although nuclear antigens, they are processed by the cell and expressed at the cell surface in the context of HLA class I molecules. Immunodeficient individuals are unable to mount an immune response against EBV epitopes, and EBV-transformed cells, which, although cytogenetically normal, are able to undergo indefinite, unrestricted proliferation, which can be life-threatening.

The role of an unbalanced cytokine response due to the uncontrolled B-cell proliferation is also being explored. Serum levels of IL-4, an inflammatory marker, are elevated in patients with PTLD as well as in healthy but immunosuppressed transplant recipients, compared with normal controls, whereas levels of IFN-a were significantly depressed.[166] Furthermore, tissues of patients with PTLD express significantly lower levels of IL-18 and IFN-g compared to tissue obtained from patients with primary IM.[167] The monokine induced by IFN-g (Mig) and the IFN-g–inducible protein-10 (IP-10) are also expressed at higher levels in tissue from patients with EBV-induced LPDs.[168]

EBV infection is associated with various forms of human lymphoid malignancies, including the sporadic and endemic forms of BL, LPDs in congenital and acquired immunodeficiency states, and posttransplant lymphomas.[169] EBV also plays a role in the development of smooth muscle tumors (i.e., leiomyomas and leiomyosarcomas) in immunocompromised hosts, and has been implicated in the pathogenesis of certain gastric carcinomas, and is recognized as the etiologic agent for the development of nasopharyngeal carcinoma.[115,170,171,172 and 173]

As shown by Purtilo in patients with XLP, primary EBV infection (e.g., IM) can become a fatal disease in patients with congenitally abnormal T-cell function.[174] Most lymphoid tumors are of B-cell phenotype, but both polyclonal and monoclonal lymphomas (of B- or T-cell phenotype) can be found, possibly representing a progression in development.

Several studies have shown that rising EBV titers in the posttransplant period can help with early identification of patients at risk for the development of PTLDs, and it has become standard of care to follow EBV titers, either by serology or by polymerase chain reaction (PCR.)[175] Furthermore, there appears to be a difference between primary EBV infection and reactivation. In a group of 50 children followed before and after heart transplant, 13 developed a PTLD, but 12 of them had evidence for primary EBV infection.[65] A similar observation has been made in pediatric liver and kidney transplant patients.[61,176] This could be the reason for the higher incidence of PTLD in children compared to adults.

In a study of 35 biopsy specimens obtained from patients with progressive generalized lymphadenopathy and HIV infection using PCR and sentence hybridization a positive correlation was seen between EBV positivity (35%) and the development of EBV-positive NHLs at another site, or the subsequent development of a lymphoma.[177] None of the patients whose biopsies were negative for EBV went on to develop a lymphoma. EBV has also been found in some patients with multicystic thymic tumors, and EBV DNA has been associated with at least 80% of lesions found in lymphocytic interstitial pneumonitis (LIP), usually in the absence of other pathogens.[178] Approximately 40% of the systemic and close to 100% of the CNS NHLs associated with HIV disease contain EBV sequences.[178,179 and 180] A third of AIDS-associated BLs show evidence for EBV infection, whereas more than 80% of AIDS-associated large cell NHLs are EBV positive.[181,182,183 and 184]

Although not true in immune-competent individuals, leiomyosarcomas from patients posttransplant as well as from HIV-infected children have been shown to be positive for EBV by *in situ* hybridization.[115,185] Quantitative PCR showed relatively high levels of EBV in tumor tissue (as many as 4.3 genome copies per cell), and discrete episomal EBV clones were present in lesions taken from different sites in the same patient, indicating simultaneous emergence of separate clones.[115] Higher numbers of EBV receptors are found on tumor cells from HIV-infected individuals compared to cells from HIV-negative patients. Jenson et al.[186] were able to demonstrate monoclonal EBV infection and replication in smooth muscle cells obtained from an HIV-infected patient with leiomyosarcoma.

### *Human Immunodeficiency Virus*

HIV-1 and HIV-2 are RNA retroviruses and belong to the family of lentiviruses. They preferentially infect macrophages, monocytes, and lymphocytes, by binding to at least two distinct chemokine receptor sites, CCR5 and CXCR4.[187,188] Infection with HIV results in both a latent and lytic infection, associated with a decline in lymphocyte numbers, especially the $CD4^+$ cells.

HIV does not appear to be directly involved in the malignant transformation of B lymphocytes, and there is a lack of integrated HIV genomic sequences into most hyperplastic lymphoid tissues or NHL. The results of PCR analysis of AIDS lymphoma tissue are consistent with the presence of HIV in infiltrating T cells within these tissues as opposed to actual HIV infection of the B-lymphoma cells.[189] However, a few cases of large cell lymphomas have been described, in which HIV integration was found upstream to the oncogene *FES/FPS*.[190] These tumors had an extremely high expression of the HIV p24 antigen.

Infection with HIV leads to the release of a number of cytokines, some of which may increase the replication of HIV, whereas others may be responsible for producing a state of B-cell growth (and hyperproliferation), activation, and differentiation.[191] HIV may, however, participate indirectly in the development of lymphomatous disease in several different ways, including the release of cytokines. Among others, several ILs (i.e., IL-1, IL-2, IL-6, IL-7, and IL-10), as well as IFN-g and tumor necrosis factor, are stimuli responsible for the proliferation and differentiation of B cells, which ultimately increase the likelihood of genetic mutations resulting in neoplasia.[192,193,194,195 and 196] For example, IL-6 has been classified as a differentiating factor for B lymphocytes, and has been shown to function as an autocrine growth factor in tumor cell lines derived from non-HIV and non-EBV–related malignant lymphomas, acting, together with IL-10, as an autocrine growth factor for

AIDS-associated primary effusion lymphomas.[197]

The HIV Tat gene is a potent transactivator of viral and cellular genes, including the gene for IL-6 and IL-10. Transgenic mice that express the Tat gene develop splenomegaly, often containing malignant B-cell clones.[198] In these mice, the messenger RNAs encoding IL-6 and IL-10 were up-regulated. In another study, Tat significantly increased the motility of AIDS-related NHL cell lines by enhancing the adhesion of lymphoma cells to the endothelium.[199] HIV infection stimulates surface expression of CD40 on endothelial that in turn triggers the preferential induction of the vascular adhesion molecule VCAM-1.[200] Extracellular Tat protein has also been found to have angiogenic properties, to promote KS, to cause normal vascular cells to migrate, and to stimulate new vessel growth.[201,202] Vascular endothelial growth factor (VEGF-A) concentrations are higher in sera of HIV-positive patients with and without KS compared to uninfected individuals.[203] Stimulation of vascular permeability by VEGF has been found to be important in the pathogenesis of AIDS-associated primary effusion lymphomas.[204]

Infection with HIV leads to an up-regulation of preexisting mechanisms. Non-immortalized peripheral B lymphocytes from EBV-positive, HIV-negative donors can be infected with HIV, and a subset will then develop enhancement of EBV DNA and RNA as well as deregulation of c-*myc* transcripts and protein.[205]

The likelihood to develop a chromosomal translocation could be increased in HIV-infected individuals due to the probable accumulation of a significant number of mutations in tumor suppressor genes that occur due to the continued proliferation of B cells secondary to immunosuppression. It has been shown for example, that the failure of p53 to permit DNA repair during the cell cycle can permit mutations to persist in successive cell generations. Progression from oligoclonality to monoclonality, associated with the occurrence of chromosomal abnormalities in immunodeficient individuals, has been demonstrated in reactive lymphadenopathy by the presence of multiple clonal B-cell expansions as multiple Ig gene rearrangements.[206,207] However, only one of these clones has been found to have a c-*myc* rearrangement, suggesting that only one clone carries the genetic abnormality associated with a B-cell lymphoma, whereas the other B-cell expansions only represent precursor lesions. Inactivation of p53 is one of the most common genetic abnormalities associated with human cancers. In HIV disease, it is exclusively associated with AIDS-BL (60%), which is more common than in sporadic or endemic BL (30%), although it is not found in AIDS-related diffuse large cell lymphomas (DLCLs) or primary effusion lymphomas.[184,208] Although deletions of 6q are common in B-cell NHL of immunocompetent hosts, it is only found in approximately 20% of AIDS-DLCLs but is absent in other AIDS-associated lymphomas.[184,209]

Translocations affecting 8q24 t(8;14)(q24;q32), the c-*myc* locus, and an Ig locus, are commonly seen in BL. Adults with AIDS-BL typically show c-*myc* activation (in 100% of cases) and often a disruption of p53 function (in 60%).[182,184,210,211] Clones of circulating peripheral lymphocyte with Ig chain and c-*myc* abnormalities can be found in HIV-positive homosexual men without a clear correlation to the subsequent development of NHL.[212] In children, the frequency of c-*myc* mutations is lower, although translocations clearly do occur. One study found known mutations of c-*myc* in only 2 of 13 small non-cleaved cell lymphomas, whereas 8 of 11 tumors showed translocations of the Ig gene/c-*myc* region.[178]

AIDS-associated DLCLs show *bcl*-6 rearrangements in 20% of cases compared to 40% of DLCLs occurring in immunocompetent hosts.[184,213] Mutations can also occur in the 5' non-coding region of *bcl*-6, and are seen in 60% of systemic NHLs, especially the AIDS-BLs and AIDS-DLCLs (70%), but less commonly in body cavity–based lymphomas (20%).[213] Expression of the bcl-6 protein varies as well between the different types of AIDS-associated NHL. It is expressed in all AIDS-BL but only in one-half of the AIDS-DLCLs, and only if there was no concurrent LMP-1 expression.[214]

***Human Herpesvirus 8 or Kaposi's Sarcoma–Associated Herpesvirus***

In 1994, a unique DNA sequence was found within KS lesions and identified as protein genes of a new gamma herpesvirus, now called *human herpesvirus 8* (HHV-8) or *Kaposi's sarcoma–associated herpesvirus*.[215] HHV-8 has been shown to be present in close to 100% of all KS lesions, and has also been found in AIDS-related body cavity NHL, lymphomatous effusions without tumor mass, and Castleman's disease, as well as some cases of multiple myeloma.[216,217,218,219] and [220] Only a few cases of childhood KS have been studied for the presence of HHV-8, but the herpes virus was present in all of them.[93] It has recently been shown that HHV-8 can be propagated from AIDS-associated KS lesions.[221]

Multicentric Castleman's disease, an atypical LPD, has been strongly linked to the presence of HHV-8, which might explain the coexistence of KS in many patients.[222,223] A correlation between the HHV-8 viral load and progression of disease has recently been established.[224] HHV-8 is more commonly found in multicentric Castleman's disease than in the form with an isolated lesion, and more frequently in HIV-infected patients, but has also been described in PTLDs.[225,226] and [227]

Primary effusion lymphomas comprise approximately 5% of all HIV-associated lymphomas, and they have a strong association with HHV-8 infection (as well as with EBV infection).[226,228,229] and [230] A recent study demonstrated the high levels of nerve growth factor are secreted in an autocrine fashion by the HHV-8–infected cells and are essential for virus maturation and survival.[231]

More than 80% of all patients with KS (HIV-positive or -negative) have specific serum antibodies to HHV-8–related antigens, often detectable before the clinical onset of KS lesions.[103,219,232,233] In the general U.S. population, approximately 25% of adults have HHV-8–related antibodies, compared to 18% in adolescents and less than 4% in children younger than 13 years of age, indicating sexual transmission as the most likely mode of infection.[234,235,236] and [237] However, in African countries such as Nigeria or Zaire, the seroprevalence is much higher (56% and 82%, respectively).[238,239] Seroprevalence for HHV-8 was also found to be increased in immunosuppressed renal allograft recipients (50%) compared to healthy adults (7%) and to reach similar levels as in an HIV-infected control group (73%).[240]

Several potential oncogenes have been identified that might play a role in the development of HHV-8–associated malignancies.[241,242] and [243] They appear to disturb both the retinoblastoma (pRb) and p53 tumor suppressor and cell cycle pathways, as well as interfere with the IFN signaling pathways.[243,244]

***Human Papillomavirus***

Malignant melanoma after renal transplant has been shown to frequently arise from precursor nevi, associated with an abnormal host response to the tumor (i.e., absence of lymphocyte and macrophage infiltrates).[245] HPV DNA, mainly belonging to the epidermodysplasia-verruciformis subgroup, is commonly found in premalignant and cancerous skin lesions of renal transplant patients. However, the prevalence of epidermodysplasia-verruciformis-HPV DNA is equally high in patients with or without a history of skin cancer, indicating that other factors contribute to the development of a malignant lesion.[246]

There is a strong correlation between infection with HPV, especially types 16 and 18, and the development of cervical or anal neoplasms.[122,127,130,132,139,246,247,248] and [249] Immune status and co-infection with HIV or other sexually acquired organisms appear to play a role as well.[122,126,130,250]

***Other Microbial or Viral Pathogens***

Several microbiologic agents have been implicated in the pathogenesis of LPDs. Mucosa-associated lymphoid tumors (MALTs), both in HIV-negative as well as in HIV-infected patients, have been shown to be associated with the presence of *Helicobacter pylori* in the gastric mucosa.[251,252,253] and [254] It is thought that the continuing inflammatory reaction and antigen exposure predisposes the patient to the development of a mucosal tumor, often associated with an accumulation of p53 abnormalities.[255]

The roles of HHV-6 and HHV-7 in the pathogenesis of LPDs are not yet completely resolved.[256,257,258,259] and [260] Infection with HHV-6, the cause of roseola infantum (exanthema subitum) is common. In the immunocompetent host, it causes a self-limited disease and then remains in its latent form. In immunocompromised patients, however, HHV-6 can cause transplant rejection, bone marrow failure, pneumonitis, or encephalitis, as well as hepatitis and various skin rashes.[260] HHV-6 is found in some lymphoproliferative processes in these patients, however, whether it is just ubiquitous or whether it has an oncogenic role is not clear. Some cases of chromosomal integration of HHV-6 into lymphoma cells have been described.[256,261] HHV-7 has selective tropism for $CD4^+$ lymphocytes and appears to be able to reactivate HHV-6. It has not yet been associated with a human disease but is suspected to have a potential role in the pathogenesis of LPDs.[257,258,262]

**Cytokines and Other Pathogenetic Factors**

Abnormalities in the expression of cytokines are commonly found in many patients with LPDs or malignancies associated with immunodeficiency, but we are only beginning to understand the complex regulatory mechanisms that are involved.

Cytokines and angiogenesis factors play a dominant role in the pathogenesis of KS. [201,202,263] KS cell growth is sustained *in vitro* by basic fibroblast growth factor, IL-1, IL-6, oncostatin M, and other cytokines. [263,264] The angiogenic basic fibroblast growth factor acts synergistically with the HIV-1 Tat protein, enhancing endothelial cell growth and type-IV collagen expression. [202] AIDS-KS cell lines also express VEGF/vascular permeability factor, as well as the receptors for VEGF (i.e., Flt-1 and KDR), indicating that VEGF is yet another autocrine growth factor for KS cells promoting neo-angiogenesis. [264]

Chemokines and variants of chemokine receptor genes influence the risk for the development of NHL in HIV-infected children and adults. The stromal cell–derived factor 1 chemokine variant is associated with a twofold increase of the NHL risk in heterozygotes and roughly a fourfold increase in homozygotes. [265] In particular, the stromal cell–derived factor 1-3' A variant was associated with BL and Burkitt's-like NHL, the same tumors that also show c- *myc* activation.

## PATHOLOGY AND TREATMENT

Tumors in immunocompromised patients often display a unique pathology. The border between “benign” cellular proliferation and overt malignancy is intertwined, as demonstrated by the LPDs and the smooth muscle tumors. The interaction between concurrent viral infections and the underlying inability of the immune system to mount an appropriate response leads to typical cytopathogenic changes. Treatment includes improvement (if possible) of the underlying immunodeficiency, treatment of concurrent viral infections, and tumor-specific treatment that is tailored to the diminished capability of these patients to tolerate aggressive interventions. A problem inherent in treating patients with immunodeficiencies and malignancies is that the risk to develop a new tumor continues as long as the immune system is abnormal. This chapter emphasizes the differences in pathology and treatment of tumors occurring in immunocompromised patients; for more detailed review of pathology and treatment, please refer to the disease-specific chapters.

### Non-Hodgkin's Lymphoma

Although most lymphomas seen in immunocompromised patients can also be found in otherwise healthy hosts, there are some distinct features. Nevertheless, an international panel of experts recently determined that no separate classification is needed for lymphoid tumors occurring in immunocompromised patients, with the exception of PTLDs (Table 25-3). [266]

| | |
|---|---|
| Early manifestations | Reactive plasmacytic hyperplasia |
| | Infectious mononucleosis-like |
| Polymorphic PTLD | Polyclonal (rare) |
| | Monoclonal |
| Monomorphic PTLD (classified according to lymphoma classification) | B-cell lymphomas |
| | Diffuse large B-cell lymphoma (immunoblastic, centroblastic, or anaplastic) |
| | Burkitt's or Burkitt's-like lymphoma |
| | Plasma cell myeloma |
| | T-cell lymphomas |
| | Peripheral T-cell lymphoma, not otherwise categorized |
| | Other types (hepatosplenic, gamma-delta, T cell/natural killer cell) |
| Other types (rare) | Hodgkin's disease–like lesions (associated with methotrexate therapy) |
| | Plasmacytoma-like lesions |

**TABLE 25-3. SUGGESTED WORLD HEALTH ORGANIZATION CLASSIFICATION OF POSTTRANSPLANTATION LYMPHOPROLIFERATIVE DISORDERS (PTLD)**

The majority (>90%) of NHLs in immunocompromised patients are high-grade B-cell malignancies. [8,97,104,152,267] Although most NHLs in HIV-infected adults and children are of B-cell origin, there is an increasing number of case reports of T-cell tumors, especially Ki-1 $^{+}$ (CD30$^{+}$) anaplastic large cell lymphomas. A similar observation has been made in NHL occurring after solid organ transplants. [64]

Extranodal sites are involved in the majority of HIV-positive patients (87%), and approximately 34% have bone marrow infiltration at the time of diagnosis. [268] Other commonly involved extranodal sites are the gastrointestinal tract, liver, CNS, lungs, and bone, but almost any organ can be affected. [91,178,268,269] Primary CNS lymphomas account for 4% to 40% of the NHLs in HIV-infected adults and have also been described in children with AIDS. [112,178] A similar incidence of primary CNS lymphomas (20% to 40%) has been reported in the posttransplant setting and some congenital immunodeficiency disorders. [8,55,65,206] Body cavity and primary effusion lymphomas are relatively recently recognized manifestations of NHL in immunocompromised hosts, but have so far only been seen in adults. [178,218,270]

#### *Systemic Non-Hodgkin's Lymphoma*

The clinical presentation of systemic NHL in the HIV-infected child is somewhat different from tumors seen in HIV-infected adults. Patients can have marked variability in age, sites of presentation, and CD4 count at diagnosis. [91] A similar variability has been observed in patients with congenital or iatrogenic immunodeficiency states. [8] Extranodal involvement (including lungs, liver, bones, and meninges) is common and may result in vague and difficult to evaluate symptoms such as refusal to walk, pleural pain, or hepatomegaly. [8,111,112,271] Nonspecific complaints, including fatigue, loss of appetite, and night sweats, are common.

When a lymphoma is suspected, a careful staging should include radiologic studies with computed tomography or magnetic resonance imaging, a gallium and bone scan, a bone marrow examination, and a lumbar puncture. Because the underlying disease (e.g., HIV infection) or its treatment (e.g., transplantation) often result in multi-organ problems as well as the use of several different drugs, it is important to assess hepatic, neurologic, renal, bone marrow, and cardiac function as best as possible before intervention.

The treatment of systemic NHL in immunocompromised patients is still evolving. In general, older (adolescent and adult) patients often have difficulties tolerating aggressive chemotherapeutic regimens. HIV-infected adults with systemic NHL have a median survival of 5 to 8 months, and only 10% to 20% remain disease-free for more than 2 years. [272,273 and 274] An initial attempt to use more intensive chemotherapy based on the more aggressive nature of the HIV-associated NHLs proved unsuccessful because of a high mortality due to opportunistic infections. [272,275] Subsequent trials using reduced-dose regimens yielded better results in regard to treatment-associated hematologic toxicity with similar disease-free survival rates. [273,276] The standard of care is still evolving, but m-BACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone) at low or standard dose levels is commonly used in adults. HIV-infected children have successfully been treated with a simpler regimen of cyclophosphamide (day 1) and methotrexate (day 10), combined with intrathecal cytarabine (days 1 and 3) and intrathecal methotrexate (day 10), administered for three cycles (unpublished results). Because BLs or Burkitt's-like lymphomas are the most common NHL type in HIV-infected children, shorter and therefore better tolerated chemotherapy regimens can be used. Regardless of the age and chemotherapy used, it is important to continue to treat the underlying HIV infection, and, if possible, to choose drugs with non-overlapping toxicities. [273,276,277 and 278]

Even in the presence of a monoclonal malignant lymphoma, it might be worthwhile to decrease the degree of immunosuppression in patients with posttransplant NHL. [279] If this alone is not sufficient, chemotherapy may be used and is usually relatively well tolerated. [64,65,280,281] A newer approach is the use of monoclonal B-cell antibodies directed against surface antigens on tumor cells, including CD20, CD21, and CD24 for the treatment of posttransplant malignant LPDs. [282,283 and 284] Rituximab (anti-CD20 antibody) is licensed in the Unites States for the treatment of NHL in adults, and preliminary results support its use in CD20-positive PTLD as well. [285,286,287 and 288] Studies are currently under way to evaluate safety and efficacy of rituximab in pediatric patients.

#### *Primary Central Nervous System Non-Hodgkin's Lymphoma*

Primary CNS lymphoma occurs both in immunocompetent and immunocompromised patients, but survival is markedly different.[289] For unknown reasons, the incidence of primary CNS NHL is not only high in patients with AIDS but also steadily increasing in immunocompetent patients, becoming one of the most common primary malignant neoplasms in the brain in adults.[290,291]

Patients with primary CNS lymphoma are generally symptomatic for several weeks before diagnosis, and may show symptoms of confusion, lethargy, memory loss, seizures, hemiparesis, or other focal neurologic signs, as well as signs suggestive of increased intracranial pressure.[289,292,293] Radiographic imaging by computed tomography or magnetic resonance imaging typically demonstrates an isodense or hypodense single lesion that enhances with contrast, although multiple lesions are present in almost one-half of the patients with AIDS, compared to only 25% of immunocompetent patients.[289,292,293] Ring enhancement is seen in approximately one-half of the patients with AIDS-associated NHL of the brain, whereas it is rare in immunocompetent hosts.[289]

The differential diagnosis of a parenchymal brain lesion in an immunocompromised patient includes neoplasm (usually lymphoma), infection (usually toxoplasmosis, but rare in children), and hemorrhage (Fig. 25-1). The clinical, histologic, and radiographic findings in children are similar to the adult population. In children, however, in whom CNS toxoplasmosis is rare, the most likely diagnosis of an intraparenchymal brain lesion is lymphoma or another brain tumor. Abnormalities of cerebrospinal fluid, with mild mononuclear pleocytosis, slightly elevated protein, and occasional decreased glucose, have been described, and malignant cells were found in approximately 25% of the patients in whom it was considered safe to perform a lumbar puncture.[289,294]

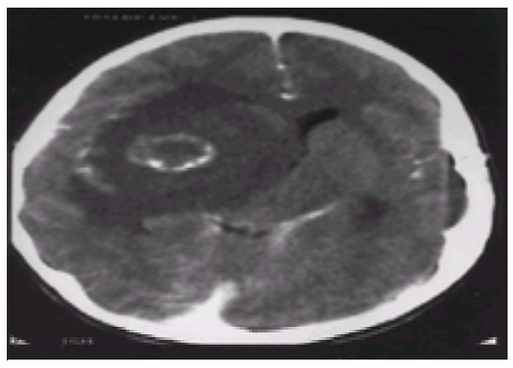


**FIGURE 25-1.** Mass lesion in the cerebrum of a 12-year-old hemophiliac. The differential diagnosis includes malignant lymphoma and central nervous system toxoplasmosis. This child responded well to treatment with pyrimethamine and sulfadiazine, the standard therapy for toxoplasmosis.

The CNS lymphomas in immunocompromised patients are intracranial intraparenchymal tumors, characterized by frequent histologic multifocality, indistinct borders, and often an angiocentric arrangement.[295] They are generally high-grade tumors, mostly large cell immunoblastic lymphomas (in 35% to 45%) and small non-cleaved lymphomas (in 25% to 36%), which is different from immunocompetent hosts in whom only 22% have high-grade morphology (18% immunoblastic and 4% small non-cleaved cell type).[289,296] Most tumors are of B-cell origin; in immunocompromised hosts, virtually 100% of all CNS NHLs are EBV positive, whereas this is rare in immunocompetent patients.[289,297]

Treatment of primary CNS lymphomas consists mainly of radiation with or without chemotherapy. Patients with AIDS who develop a CNS NHL often have far advanced HIV disease, making a more aggressive intervention difficult.[271,272,289,294,298]

### Hodgkin's Disease

Clinically aggressive types of HD are more common in HIV-infected patients and probably in other immunocompromised populations as well.[299] More than 80% of HIV-infected patients with HD present with systemic symptoms, and disseminated stage III or IV disease occurs in approximately 75% to 90% of patients: bone marrow involvement is common (in 40% to 50%).[110] In an Italian study that compared the presentation of HD in infected and non–HIV-infected adults, HIV-associated HD occurred in younger patients (29 years versus 38 years); had a male predominance (90% versus 56%); and presented more often with stage IV disease (63% versus 29%), B symptoms (77% versus 35%), and extranodal disease (63% versus 29%).[300] In contrast to NHL, there is no clear correlation between the occurrence of HD and CD4 counts. Most patients with HIV-related HD did not have a prior diagnosis of AIDS, but a substantial proportion had a history of generalized lymphadenopathy (in 36% to 83% of patients).[110,300,301]

Mixed cellularity and lymphocyte depletion are the most common histologies in HIV-infected patients (66%), compared to lymphocyte predominance and nodular-sclerosing types in HIV-negative patients (71%).[110] Reed-Sternberg cells, the presumed neoplastic cells of HD, are more prominent in HIV-related HD.[302] Evidence for latent EBV infection is demonstrated in the majority (78%) of patients with HIV-associated HD, and 80% of these tumors contain monoclonal EBV genomes.[300]

The optimal chemotherapy for HD in immunocompromised patients has not yet been established. HIV-negative patients with stage III and IV disease achieve cure rates of more than 70% with either a mechlorethamine, vincristine, procarbazine, and prednisone regimen (MOPP), or a doxorubicin, bleomycin, vinblastine, and dacarbazine regimen (ABVD); however, HIV-infected patients have a much poorer outcome.[110,303,304] In a recent study of epirubicin, bleomycin, vinblastine and prednisone, combined with antiretroviral therapy and granulocyte colony-stimulating factor, the median survival was 16 months, with a survival rate of 32%.[304]

### Lymphoproliferative Disorders

LPDs in immunocompromised patients are commonly of the B-cell type, although T-cell tumors have been described as well. They can range from asymptomatic hypergammaglobulinemia (especially in HIV-infected children) to generalized lymphadenopathy with hepatosplenomegaly. The transition to a malignant process is not clearly defined, because even a low-grade, polyclonal process can become life-threatening due to its location. Polyclonal LPDs, especially if EBV associated, can evolve into a rapidly progressive monoclonal process.[305] However, as shown in PTLDs, decreasing the degree of immunosuppression can cause regression in some patients even if they have monoclonal tumors.[279,281]

#### *Lymphadenopathy and Hypergammaglobulinemia*

The lymph node architecture is preserved in HIV-related lymphadenopathy with hypergammaglobulinemia and typically shows polyclonal follicular (B-cell) hyperplasia.[112,306] However, hyperplastic lymph nodes can occasionally contain occult clonal B-cell populations.[207] This lymphadenopathy either resolves spontaneously or responds to initiation or optimization of antiretroviral therapy. Atypical lymphoid hyperplasia or reactive lymphoid hyperplasia are the most common lymphoproliferative lesions in patients with congenital immunodeficiencies such as CVID, WAS, or SCID.[41,268]

A monoclonal gammopathy can precede the development of an EBV-associated PTLD. In a study of 201 patients after liver transplant, 57 (28%) showed a monoclonal urine or serum protein after transplantation, including five of seven patients (71%) who developed a PTLD and 27% of patients who did not.[307] The authors identified cytomegalovirus (CMV) positivity or postoperative CMV infection as the major risk factors ($p$ <.02).

#### *Lymphocytic Interstitial Pneumonitis and Diffuse Infiltrative Lymphocytosis*

LIP is a typical finding associated with HIV infection in children.[306,308] It can remain a radiographic finding only or become clinically problematic because of the development of oxygen dependency. Histologically, both B and T cells (especially CD8+ T cells) are present in either nodular or interstitial distribution.[308]

A diffuse infiltrative lymphocytosis syndrome, consisting of bilateral parotid enlargement, cystic enlargement of the salivary or lacrimal glands, has been described in HIV-infected adults and children.[112,309,310] The patients described by Kontny et al.[117] who presented with multiple cystic lesions in their grossly enlarged thymus, may also belong in the same group (Fig. 25-2).

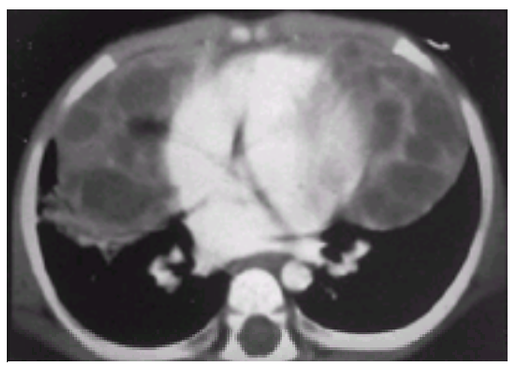


**FIGURE 25-2.** Cystic mediastinal mass in a child with human immunodeficiency virus infection. Histologically, the mass was a polyclonal, Epstein-Barr virus–positive lymphoproliferative process within the thymus, consisting of B and T cells forming distinct lymphoid follicles with germinal centers.

### *Mucosa-Associated Lymphoid Tumors*

MALT lymphomas are at the interface between LPDs and malignant lymphoid tumors and tend to occur in the lungs, the gastrointestinal tract, and parotid and salivary glands.[178,254,311,312] They can occur both in immunocompetent and immunocompromised patients. MALT lymphomas comprised 10% of all NHLs in our series of HIV-infected children with tumors and have also been described in posttransplantation patients.[91,313] Several cases of pulmonary MALT have been associated with LIP, and a pathogenetic link is currently being investigated.[254,314] Immunoproliferative small intestinal disease is another form of MALT especially prevalent in the Mediterranean region, and has been described in some children as well.[315]

MALT lymphomas are monoclonal B-cell tumors that can display cytogenetic abnormalities (e.g., trisomy 3), and may be heterogeneous for *bcl*-2 expression and p53 mutations, which are markers for blocked apoptosis.[254,312,316,317] A translocation between chromosomes 11 and 18, t(11;18)(q21;q21), is commonly found in MALT lymphomas.[318,319] Most MALT lymphomas are low-grade tumors and remain localized at their sites of origin for many years, although transformation to high-grade malignancies can occur.[254,320,321]

Surgical excision, especially of pulmonary lesions, is often all that is needed for successful treatment, and survival seems to be favorable because all children from our series are alive 17 to 36 months after cancer diagnosis.[91,312] Gastric MALT tumors have been shown to be strongly linked to a local infection with *H. pylori* and often respond to antimicrobial therapy (e.g., amoxicillin and metronidazole) combined with omeprazole or bismuth, and some patients with immunoproliferative small intestinal disease respond to treatment with tetracyclines.[252,253,315,322] If this does not eradicate the tumor, single-agent chemotherapy with oral cyclophosphamide or chlorambucil can be tried.[323]

### *Posttransplant Lymphoproliferative Disorder*

As mentioned, lymphoproliferative processes in the posttransplant period range from lymphoid hyperplasia to malignant NHL. Although the majority of PTLDs are of B-cell origin, occasional T-cell tumors have been observed, especially in late-occurring tumors.[71] Simultaneous occurrence of clonally distinct lesions is possible.[324] Non-lymphomatous PTLD can present as plasmacytic hyperplasia or a polymorphic lymphoid process (Table 25-3). Plasmacytic hyperplasia is often confined to the tonsils or adenoids, whereas polymorphic lesions can occur in lymph nodes or extranodular sites, including the allograft.[58,65,67,282,325,326,327] and [328] Neither lesion shows any alteration in oncogene or tumor suppressor gene expression, and most patients respond to a decrease in immunosuppression.[328] This regression might be associated with an expansion of $CD8^+$ T cells.[329] Tonsillar or adenoid lesions can often be treated with excision alone.[330]

Decreasing the degree of immunosuppression often results in a marked regression of a PTLD. The use of intravenous Ig and IFN-a have been effective in some patients, but no controlled studies have been performed.[331,332] and [333] Although acyclovir or ganciclovir are commonly used in patients with EBV-related PTLD, their efficacy is doubtful, because EBV in its latent form is less accessible to therapy. Furthermore, the EBV thymidine kinase does not phosphorylate the two drugs, a necessary step for activation.[334] Several new and exciting therapeutic approaches are being studied, including immunotherapy with infusion of donor T lymphocytes, adoptive transfer of gene-modified virus-specific T lymphocytes, and the use of monoclonal antibodies such as rituximab (anti CD20).[283,284,335,336] and [337]

### *Castleman's Disease*

Castleman's disease, also called *giant lymph node hyperplasia* or *angiofollicular hyperplasia*, is rare in children.[338] The disease is usually localized. A hyalinovascular type is seen in 54%, whereas the plasma cell type is encountered in 24% of cases.[338] Multicentric Castleman's disease is seen more commonly in HIV-infected adults and other immunocompromised patients.[82,223] This systemic lymphoproliferative process, probably originating from plasma cells, often presents with hypergammaglobulinemia, has a frequent association with KS (in 75% of cases), and is, in some patients, associated with the development of NHL at a later time.[223,227,339,340] Like KS, it has a strong association with HHV-8 infection.[222,225,226] and [227,241,341] In the immunocompetent patient, treatment may consist of surgery only, or include steroids and other immunosuppressive agents, whereas low-dose and single-agent chemotherapy (mainly vinblastine) has shown some success in HIV-infected patients.[223,342]

### *Angioimmunoblastic Lymphadenopathy with Dysproteinemia*

Angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) is a rare systemic LPD, presenting with diffuse lymphadenopathy, hepatosplenomegaly, fever, pulmonary infiltrates, or pleural effusions, as well as autoimmune hemolytic anemia with positive Coomb's test and a polyclonal hypergammaglobulinemia.[343,344] This disease is extremely rare in childhood, but a few cases have been described.[345,346] The lymph node architecture shows effacement, prominent neovascularization, and a diffuse infiltration by immunoblasts and plasma cells. The majority of AILDs are clonal T-cell processes, with prominent CD4 positivity.[344] Clonal abnormalities, especially trisomies 3, 5, and X, have been described.[347] Spontaneous regression of the lymphadenopathy has been observed, but AILD is often a rapidly progressive disease. Treatment has not been very successful, and only approximately 25% of patients achieve prolonged remissions even with aggressive multi-agent chemotherapy.[344]

### *Lymphomatoid Granulomatosis*

Lymphomatoid granulomatosis is an LPD that often presents in extranodal sites, especially the lungs, liver, kidneys, skin, and CNS.[348,349] It can occur both in immunocompetent and immunocompromised patients, but has only rarely been seen in children.[350,351,352] and [353] Histologically, lymphomatoid granulomatosis is an EBV-positive B-cell proliferation associated with an exuberant T-cell reaction.[349] Approximately 60% of cases show clonal rearrangements of the VDJ region. Spontaneous regression is possible, but a rapidly fatal course with development of a malignant lymphoma is more common. Treatments include chemotherapy with steroids and cyclophosphamide, or a trial with IFN-a.[349,354]

### *Lymphomatoid Papulosis*

A rare cutaneous T-cell LPD, lymphomatoid papulosis, is mainly seen in elderly adults, but a few pediatric cases have been described. [355,356,357 and 358] Patients may experience a waxing and waning course, with involuting and recurring cutaneous papules, plaques, and nodules, sometimes followed by the emergence of a cutaneous $CD30^+$ NHL.[356,358,359]

### *Autoimmune Lymphoproliferative Syndrome*

Massive nonmalignant lymphadenopathy, a variety of autoimmune manifestations, and an expanded population of $TCR^-CD3^+CD4^-CD8^-$ (double negative) lymphocytes characterize the recently recognized autoimmune lymphoproliferative syndrome (ALPS). [360,361,362,363,364 and 365] Patients typically present with lymphadenopathy, splenomegaly, autoimmune hemolytic anemia, autoimmune neutropenia, and thrombocytopenia. Lymph nodes show marked paracortical hyperplasia due to infiltration by plasma cells and double-negative lymphocytes. [362]

Most patients with autoimmune lymphoproliferative syndrome have been found to have heterozygous mutations in the lymphocyte surface protein Fas, leading to impairment of the apoptotic pathway.[360,361,364,365 and 366] A similar clinical phenotype is typically seen in *lpr* and *gld* mice, in which homozygous mutations of the Fas or FasL gene impair Fas-induced apoptosis, leading to lymphoproliferation and generalized autoimmune disease. [367]

Splenectomy may be required to control the autoimmune thrombocytopenia and hemolytic anemia. However, the benefit from this procedure may be only transient, and postsplenectomy sepsis is not uncommon in these patients. Intermittent treatment with steroids for severe episodes of hemolysis or thrombocytopenia have also been tried.[362]

## Kaposi's Sarcoma

Three distinct forms of KS have been recognized, including an endemic African form, KS occurring mainly in elderly men in the Mediterranean regions, and KS observed in patients after transplantation or affected with HIV disease ( Table 25-4).[12,368,369]

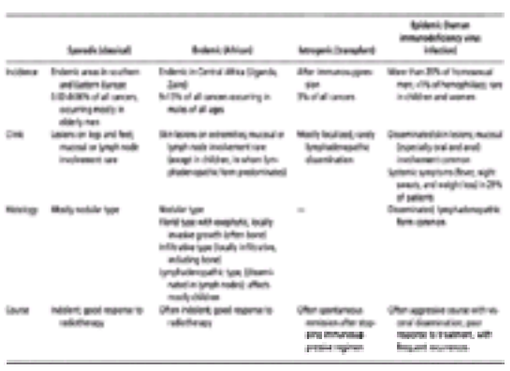


**TABLE 25-4. CLINICAL AND PATHOLOGIC FEATURES OF KAPOSI'S SARCOMA**

AIDS-associated KS occurs in a mucocutaneous and a lymphadenopathic form. Cutaneous lesions can occur in many locations, including the palms and soles, eyelids, scalp, and auditory canal. Mucous lesions can easily be detected in the oral cavity or during endoscopy. A severe but rare presentation of the mucocutaneous variant is involvement of the lungs, leading to respiratory distress. [370] The lesions have a purple or brown color, can be macular, plaque-like or nodular, and are often associated with edema due to venous congestion.[93,368] The lymphadenopathic form is the predominant form in Africa.[93,94] Three distribution patterns have been observed among 100 childhood KS cases in Uganda: an oro-facial distribution (in 79 patients) with regional (86%) or generalized (60%) lymphadenopathy and variable skin involvement (39%); an inguinal-genital distribution (in 13 children) with inguinal lymphadenopathy (100%), skin (57%), and ano-genital (14%) involvement; and a less common pattern with solitary tumors in the extremities or visceral organs (8%). [93]

KS presents as a proliferation of spindle cells surrounded by a network of reticulin and collagen fibers, as well as more or less extensive vascularization. KS cells are positive for a wide range of markers, including vimentin, von Willebrand factor, macrophage-specific CD68 and CD14, and smooth muscle (e.g., alpha-actin). [101] KS-like spindle cells have been cultured from peripheral blood, and some data support a monoclonal origin of multicentric KS lesions. [371,372]

The Oncology Committee of the AIDS Clinical Trials Group has specified criteria for the evaluation, staging, and response determination in AIDS-associated KS. [373] The staging system used by the AIDS Clinical Trials Group considers extent of the tumor, immune status (CD4 count), and signs of systemic illness to categorize patients into good and poor risk groups. Untreated KS, especially of the lymphadenopathic form or in the presence of involvement of internal organs, usually follows a rapidly progressive course.[374] In adults, KS has been treated with a variety of modalities, including local or systemic chemotherapy, radiotherapy, antiviral treatment, and, more recently, anti-angiogenesis therapy. There is limited information available regarding the treatment of children with KS. [93]

## Smooth Muscle Tumors

In children without immune compromise, leiomyosarcomas are most commonly found in the retroperitoneum and gastrointestinal tract. [375,376,377 and 378] In contrast, in HIV-infected children and otherwise immunocompromised patients, unusual localizations, such as the subcutis, spleen, pleural space, adrenal glands, lungs, and even the brain, have been described.[91,113,115,116,121,185,379,380,381 and 382] As previously mentioned, latent EBV infection is found in leiomyosarcomas from immunocompromised patients but not in tumors from otherwise healthy people.[115,119,185]

The course of disease is highly variable, with indolent tumors (more likely leiomyomas) often detected as incidental findings on routine radiographic examinations or at autopsy. However, some children present with very aggressive, widely disseminated tumors. Leiomyomas and leiomyosarcomas can be found to occur concurrently in the same patient.[379] However, smooth muscle tumors are in general not very sensitive to treatment. Decreasing the immunosuppression does not provide a clear benefit in the case of smooth muscle tumors. Local excision is the first line of therapy in non–HIV-infected children followed by chemotherapy with or without radiotherapy (see Chapter 34). Chemotherapy commonly consists of 12 months of cycles of vincristine, adriamycin, cyclophosphamide, and dactinomycin, alternating with ifosfamide and etoposide. Such a regimen is too intensive for the vast majority of immunocompromised children, but novel approaches using anti-angiogenesis drugs or methods of affecting the underlying EBV infection should be explored.

## Cervical and Anal Neoplasms

Cervical malignancies are usually detected by visual or colposcopic examination (with Pap smear) and present as multifocal lesions, not uncommonly extending to adjacent areas of the vagina, vulva, and anus.[122] Anal cancer can occur in both men and women, and the diagnosis is made by cytology, although the sensitivity of this test appears to be lower than for cervical cancer. Cervical intraepithelial neoplasia is considered a precursor lesion for invasive disease; however, this correlation is less well established for anal squamous intraepithelial lesions. [247,249] Immunocompromised patients who present with a more advanced stage of disease tend to follow a clinically aggressive course. [125,132]

The best intervention is clearly to regularly screen patients at risk, including sexually active adolescents. A discussion of the treatment options for cervical and anal malignancies is beyond the scope of this chapter. In the HIV-infected patient, it may be of benefit to improve the antiretroviral treatment in the hope of improving local immune surveillance mechanisms.[383] Both cervical and anal intraepithelial lesions that present as local disease are amenable to local excision. However, patients with more advanced disease often respond poorly to therapy.

### Skin Cancers

Squamous and basal cell carcinomas occur in a substantial number of adult patients who have undergone bone marrow or solid organ transplants. Not surprisingly, patients with fair skin and blue eyes have a higher risk for the development of skin cancers, because most lesions occur in sun-exposed areas. [384] The ratio of squamous cell carcinomas to basal cell carcinomas is reversed compared to the general population. Carcinomas of the skin and lip were the second most common tumors seen in pediatric transplant recipients. [55]

Cutaneous malignancies have a tendency to aggressive behavior, manifested by local invasion, regional metastases at diagnosis, and regional or systemic relapse after therapy.[385] The most common aggressive tumor is a poorly differentiated squamous cell carcinoma (55%) and malignant melanoma (30%). Malignant melanoma is more common in patients undergoing transplants during childhood (12% of all skin cancers, compared to 5% in adults). Furthermore, lip malignancies were twice as common in children than in adults (23% versus 12% of all tumors). [55]

### Other Malignancies

A variety of other tumors have been described in patients with weakened immune surveillance. Whether their incidence is truly increased and pathogenically linked to the immunosuppression remains to be seen.

A number of miscellaneous tumors have been reported to occur in HIV-infected children, including leukemia, Ewing's sarcoma, rhabdomyosarcoma, and ependymoblastoma.[91,296,386,387,388 and 389] As HIV-infected children survive longer and in better health, it is possible that more will develop the “normal” pediatric malignancies in the future, especially leukemia and brain tumors.

Nephrogenic adenoma of the bladder has been described in renal transplant patients and is presumably due to mechanical trauma, chemical and irradiation-induced damage, as well as viral and bacterial pathogens. In a study of seven renal allograft recipients, no correlation with immunosuppression, CMV infection, or ganciclovir therapy was found, and none of the lesions became malignant, although a recurrence was detected in five patients. [390]

Renal cortical neoplasms were found at autopsy in 32 of 1,325 solid organ transplant patients, almost 50% in renal transplant recipients (only one in an allografted kidney).[391] This represents a ninefold increased risk compared to an age-matched control population, but was mostly evident in long-term survivors. These tumors are often small and not clinically detectable. Their significance is unclear, and their pathogenesis remains to be elucidated (e.g., a history of prolonged dialysis in renal transplant patients or the degree and kind of immunosuppression).

## CONCLUSIONS

LPDs are diseases at the borderline between benign and malignant stages. An underlying immunodeficiency, whether congenital, iatrogenic, or acquired, increases the risk to develop an LPD. However, immunocompromised patients are also at risk for nonlymphoid malignancies, partly due to the fact that they are not able to contain otherwise harmless infections, such as infection with EBV, HHV-8, or other viruses. It is likely that the number of patients who develop such diseases will increase in the future, due to the still expanding AIDS epidemic in adults and children in developing nations, as well as the more prevalent use of transplantation as a therapeutic modality. These diseases present a unique opportunity to study the pathogenesis of neoplastic transformations, and are a perfect target for the evaluation of noninvasive therapies, such as anti-angiogenic drugs or cytokine regulators.

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26

# HISTIOCYTOSES

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## INTRODUCTION

The childhood histiocytoses are a rare and diverse group of disorders that have presented great difficulties for pediatricians in diagnosis and treatment. This has been true for nearly a century, since the first clear description of a childhood histiocytosis, Hand-Schüller-Christian disease. [1] The confusion surrounding the childhood histiocytoses is exemplified by the term coined for one group of these disorders, *histiocytosis X*. This term, with X standing for unknown, was proposed by Lichtenstein[2] in 1953 to underscore the lack of understanding of these disorders. Likewise, the rarity of the histiocytoses has prevented adequate epidemiologic studies to date. The only form of histiocytosis that has a definite genetic component is familial erythrophagocytic lymphohistiocytosis (FEL).

The forms of histiocytosis are frequently difficult to distinguish on a clinical basis. Yet they must be unequivocally differentiated from one another because they require different treatment approaches. Correct and complete pathologic diagnosis is essential. Paradoxically, although the diagnosis and treatment of these disorders are frequently relegated to the pediatric oncologist, the majority of the histiocytoses are not malignant diseases. The actual distinction between malignant and nonmalignant forms of childhood histiocytosis has been difficult in the past. This confusion undoubtedly has led to suboptimal treatment in some cases. In fact, the prognosis of many of the childhood histiocytoses has been considered with more pessimism than is justified. Formulation of an appropriate treatment plan rests on the ability to distinguish among the various severe forms of childhood histiocytosis and to establish an accurate diagnosis. This requires an understanding of the biologic features of this group of diseases and their relationship with the normal histiocytic subsets of the reticuloendothelial system.

## BIOLOGY

The historical association of several childhood diseases and syndromes into the single category of histiocytosis resulted from histopathologic findings that, at least at a superficial level, appeared to be common to all these diseases. The term *histiocytosis* literally signifies an increase in the number of histiocytes, or mononuclear phagocytic cells of bone marrow origin. The diseases and syndromes included under histiocytosis are characterized by an infiltration and accumulation of cells of the monocyte-macrophage series in the involved tissues. Not all diseases in which histiocytic infiltrations are prominent are classified as histiocytoses, however. In some instances, histiocytic infiltration represents a secondary rather than a primary process and one in which the primary process has been clearly defined. Some examples include graft-versus-host disease, X-linked lymphoproliferative syndrome, and certain inherited lipidoses. This implies that in the future, diseases currently classified as histiocytoses of unknown etiology may be found to have a cause that establishes the histiocytic infiltration as a secondary process, not a primary one.

An understanding of the histiocytoses requires understanding of the normal histiocyte. The origin of normal histiocytes is the bone marrow, and the differentiation of these cells occurs according to the following sequence. The most primitive precursor of the tissue macrophage is the uncommitted stem cell, found in the bone marrow. Current theory suggests that this stem cell undergoes one differentiation step to become a stem cell committed to further development in the granulocyte-macrophage series. On exposure to colony-stimulating factors, soluble factors promoting bone marrow proliferation and differentiation, the committed stem cell further differentiates into a colony-forming cell or a myeloblast-monoblast. As the next step, this precursor cell matures into the monocyte, which is found in the peripheral blood. This circulating monocyte then undergoes terminal differentiation into cells that are found in essentially every organ of the body. These cells are known by various names according to their tissue location or specialized function (e.g., bone marrow macrophages, Kupffer's cells in the liver, and Langerhans' cells in the skin).

The cells of the histiocytic system can be classified into two major subsets thought to be derived from the same bone marrow precursor: antigen-processing or phagocytic cells, and antigen-presenting or dendritic cells ( Table 26-1).[3,4,5,6 and 7] Dendritic cells are generally not phagocytic and have as their major function the presentation of antigen to both T and B lymphocytes. Follicular dendritic reticulum cells (FDCs) are found in lymph node follicles and present antigen to B lymphocytes. FDCs do not express CD45 (leukocyte common antigen) and may be of stromal, rather than hematopoietic, origin. Interdigitating reticulum cells (IRCs) and Langerhans' cells are functionally related and both present antigen to T lymphocytes. Langerhans' cells are found primarily in the skin but are also present in other organs. They are rare in unstimulated lymph nodes, but their numbers increase in certain reactive conditions. IRCs are found throughout the paracortex of normal and reactive lymph nodes. By incubation with the cytokines granulocyte-macrophage colony-stimulating factor and tumor necrosis factor-a, CD34-positive bone marrow stem cells can be caused to differentiate into Langerhans' cells.[8] Although immunoreactivity of normal Langerhans' cells is well defined, much less is known about their dynamic functional properties. What is known is that they present antigen. Whether they also either release or respond to cytokines is a question that is of more than theoretical interest, because dysregulation of cellular interactions is likely responsible for the pathogenesis of Langerhans' cell histiocytosis (LCH). Therefore, identifying the nature of abnormal cytokine interactions may allow new and specific therapeutic approaches to be developed. In fact, it is now known that the lesional Langerhans' cells in LCH both release and respond to various cytokines (in turn released by other cells, such as T lymphocytes, in the lesions), creating a veritable cytokine storm in the lesion.[9]

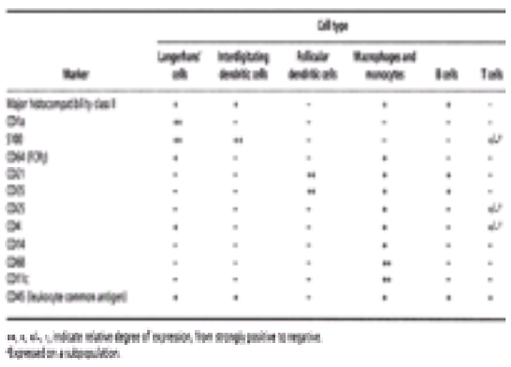
| Antigen-processing cells (phagocytic) | Antigen-presenting cells (dendritic) |
|---|---|
| Tissue macrophages | Follicular dendritic reticulum cell (lymph node follicle) |
| Monocytes | Interdigitating reticulum cell (lymph node paracortex) |
| Tingible body macrophages (lymph node follicle) | Langerhans' cell (skin, etc.) |
| Sinusoidal histiocytes | |
| Epithelioid histiocytes | |

**TABLE 26-1. HISTIOCYTIC AND RETICULUM CELL SUBSETS**

Phenotypically, dendritic cells lack the abundant lysosomal enzymes characteristic of phagocytic macrophages.[10] Both IRCs and Langerhans' cells have small amounts of lysosomal enzyme activity for acid phosphatase and nonspecific esterase. The reactivity generally has a punctate perinuclear distinction and is localized

to the Golgi region. FDCs lack lysosomal enzyme activity. All three dendritic cell types, however, manifest enzyme activity for adenosine triphosphatase.

The dendritic cell types have strong reactivity for HLA-DR, or major histocompatibility class II antigens, on their cell surface membranes ( Table 26-2).[11] Class II antigens play an important role in the presentation of antigen to T and B lymphocytes (see Chapter 5). The CD4-positive subpopulation of T cells recognizes antigen in the context of class II antigens.[11] In contrast, the CD8-positive T-cell subset recognizes antigen in the context of MHC class I antigens.



| Marker | Cell type | | | | | |
|---|---|---|---|---|---|---|
| | Langerhans' cells | Interdigitating dendritic cells | Follicular dendritic cells | Macrophages and monocytes | B cells | T cells |
| Major histocompatibility class II | + | + | - | + | + | - |
| CD1a | ++ | - | - | - | - | - |
| S100 | ++ | ++ | - | - | - | +/-* |
| CD68 (KP1) | + | - | - | + | - | - |
| CD21 | - | - | ++ | + | + | - |
| CD35 | - | - | ++ | + | + | - |
| CD57 | - | - | - | + | - | +/-* |
| CD4 | + | - | - | + | - | +/-* |
| CD14 | - | - | - | + | - | - |
| CD68 | - | - | - | ++ | - | - |
| CDF1c | - | - | - | ++ | - | - |
| CD45 (leukocyte common antigen) | + | + | - | + | + | + |

++, +, +/-, -, indicate relative degree of expression, from strongly positive to negative.
*Expressed on a subpopulation.

**TABLE 26-2. IMMUNOPHENOTYPIC MARKERS OF HISTIOCYTES AND DENDRITIC CELL SUBSETS**

IRCs and Langerhans' cells manifest immunoglobulin G (IgG) Fc receptors and, under some conditions, manifest complement receptors.[13] FDCs strongly express complement receptors (both CR1 or CD35 and CR2 or CD21). FRC is a specific monoclonal antibody that reacts exclusively with FDCs in frozen sections and is useful in their identification in normal and neoplastic lymph nodes ( Table 26-2). IRCs and Langerhans' cells are characterized by strong reactivity for S100 protein, which is totally lacking on FDCs. Smaller amounts of S100 immunoreactivity can be identified in activated monocytes and macrophages.[14]

Langerhans' cells demonstrate immunoreactivity for the CD1a antigen ( Table 26-2).[15] A recently described antibody can detect CD1a in paraffin sections and can thereby aid in diagnosis.[16] This antigen is also expressed on cortical thymocytes. IRCs generally lack the CD1a antigen, but whether CD1a immunoreactivity can be induced on IRCs in certain reactive conditions is controversial. The CD1a immunoreactive cells that appear in dermatopathic lymphadenitis may represent infiltrating Langerhans' cells derived from outside the lymph node, or CD1a immunoreactivity induced on resident IRCs. The normal Langerhans' cell is a resident of the epidermis. Originating from bone marrow precursors (CD34-positive) and possibly maturing or differentiating under the influence of granulocyte-macrophage colony-stimulating factor and tumor necrosis factor-a, this cell is the portal of entry for antigens. After antigenic stimulation, the cell migrates from the epidermis by the afferent lymphatics to the paracortical zone of regional lymph nodes and presents antigen to CD4-positive T cells. LCH cells are strongly reactive for lymphocyte function-associated antigen-3 and intercellular adhesion molecule-1, in contrast to normal epidermal Langerhans' cells, which are not. These findings suggest that abnormal homing of Langerhans' cells may be a component of the disease pathogenesis.[17]

Langerhans' cells have characteristic ultrastructural organelles known as *Birbeck granules*.[18] These rod-shaped structures of variable length contain a central striation and a vesicular expansion, which, when viewed in the appropriate cross section, resembles a racket. Birbeck granules may represent invaginations of the cell membrane and are hypothesized to result from external antigenic stimulation of the dendritic cells, with the granules related to the antigen-processing function of these cells. The granules could also represent exocytosis of intracellular vesicles, however. Which of these explanations is correct has not yet been resolved, and the function of the Birbeck granule is unknown. These granules sometimes appear as rods. When similar structures are seen by electron microscopy, measurement of the diameter of the rod defines the Birbeck granule and excludes possible errors such as the detection of viral particles. FDCs strongly express complement receptors (both CR1 or CD35 and CR2 or CD21). They also are distinguished by staining for the dendritic reticular cell antigen, clusterin, and with the monoclonal antibody CNA.42, none of which are found on macrophages, IRCs, or Langerhans' cells.[19] Finally, these antigen-presenting cell populations of all types in general lack many of the antigens expressed on monocytes and macrophages described later in this chapter.

Tissue histiocytes or macrophages are found in all the major compartments of the normal lymph node. Sinus histiocytes are the principal cells in the phagocytosis of foreign particulate matter and are located predominantly within lymph node sinuses. Tingible bodies macrophages are phagocytic cells found in normal germinal centers. In the presence of a florid follicular hyperplasia, these cells become more numerous. They frequently contain karyorrhectic nuclear debris, derived from apoptosis or karyorrhexis of proliferating germinal center cells. Tingible bodies macrophages are also conspicuous in high-grade malignant lymphomas and are especially prominent in Burkitt's lymphoma, in which they have been described as demonstrating a starry-sky pattern (see Chapter 24). Their role in these tumors is the same as that in the normal lymph node, and they contain apoptotic debris derived from dying lymphoid cells.

Tissue macrophages or histiocytes are also prominent in the paracortex of lymph nodes. In paracortical lymphoid hyperplasias, such as viral lymphadenitis, these histiocytes become prominent and produce a mottled pattern. Epithelioid histiocytes and multinucleated giant cells are associated with granulomatous lesions involving lymph nodes.

The macrophages of the reticuloendothelial system share many enzyme histochemical and immunophenotypic characteristics. They have abundant and diffuse activity for lysosomal enzymes, including acid phosphatase and nonspecific esterase.[20,21] As stated previously, all of these cells can also demonstrate phagocytosis under appropriate conditions. HLA-DR antigens, complement receptors, and receptors for the Fc fragment of IgG are common to all macrophages in lymph nodes. Activity for lysozyme and alpha$_1$-antitrypsin can be seen in all the foregoing subtypes as well, but this activity is most prominent in epithelioid histiocytes.[22] Activity for lysozyme decreases abruptly with phagocytosis, presumably because of its loss into lysosomal vacuoles.

Various monoclonal antibodies have been derived that react with monocytes and macrophages.[23,24] None of these reagents is specific for the mononuclear phagocytic system, however, and all have been shown to react with other hematopoietic cells as well, including myeloid cells, T cells, and B cells (see Chapter 6). Therefore, in the diagnosis of the histiocytoses one must interpret immunostaining and other monoclonal antibody diagnostic methods strictly and only in the context of the histologic or histopathologic features of a given lesion. The CD11c antigen present on monocytes and macrophages is absent from normal T and B lymphocytes but is expressed on the cells of hairy cell leukemia (a B-cell lymphoproliferative disorder), in low-grade B-cell lymphoproliferative disorders, and in some T-cell lymphomas.[25] Similarly, the CD14 antigen is present on monocytes and macrophages, but it can also be seen occasionally on normal and neoplastic B lymphocytes.

Cross-reactivities with T cells are present as well. For example, the CD4 antigen characteristic of the so-called helper T-cell subset is found in normal monocytes and macrophages.[26] Receptors for interleukin-2 (IL-2R; CD25), in addition to being found on activated T lymphocytes, are found on normal monocytes and macrophages.[27,28] Transferrin receptors (CD71), normally a feature of activated or proliferating hematopoietic and nonhematopoietic cells, are strongly expressed on monocytes and macrophages, even if these cells do not appear to be undergoing cellular proliferation.[29]

The monoclonal antibody KP-1, to CD68, is a useful antibody to detect macrophages in both frozen and paraffin sections.[30] CD68 is also expressed in some immune myeloid cells and is positive in granulocyte sarcomas.

That all the macrophage and reticulum cell subsets express the leukocyte common antigen detected by CD45 antibodies attests to their common hematopoietic origin. Many of the antigens expressed on macrophages are involved in triggering of phagocytosis, killing, adhesion and activation. These include CD11a, CD11b, CD11c, CD13, CD14, CD15, CD16 (FcR), CD17, CD18, CD25, CD31, CD32 (FCR), CD35, CD36, CD64, and CD68.

## PATHOPHYSIOLOGY

Several theories have been advanced to explain the pathophysiology of the four major severe childhood histiocytoses. In the case of histiocytosis X (now correctly called *LCH*; see subsequent discussion), it is suspected that immunologic stimulation of a normal antigen-presenting cell, the Langerhans' cell, continues in an uncontrolled manner, resulting in the proliferation and accumulation of these cells. That this results in disordered immunoregulation that may be central to the disease

process is suggested by findings of defective immunologic function in patients with LCH. [31] These patients showed autocytotoxicity, or destruction of their own fibroblasts and antibody-coated erythrocytes by their own effector cells *in vitro*. These immunologic findings were associated with abnormal thymic histology.[31,32] Furthermore, the administration of thymic extract was associated with clinical improvement and reversal of the defective immunologic responses *in vitro*. A continuing issue of controversy is whether LCH is a malignant disease. The demonstration of a clonal origin of lesional LCH cells suggested this possibility, [33,34] although clonality is certainly not a *sine qua non* of malignancy, and these cells are not aneuploid. Finally, that there may be genetic factors contributing to the pathophysiology of LCH is suggested by new evidence of familial clustering of LCH and high LCH concordance rates in presumed monozygotic twins. [35]

Histiocytic reactions that are secondary to known causes and do not result in a self-perpetuating hemophagocytic reaction would be expected to disappear on resolution of the underlying disease process. As discussed in detail later in this chapter, such is the case in the infection-associated hemophagocytic syndrome (IAHS). Thus, in this syndrome, the macrophage is possibly reacting to a foreign antigen adsorbed onto the formed blood elements, including erythrocytes.

An alternate explanation is that the hemophagocytic syndrome may be secondary to excessive cytokine or lymphokine production by normal or neoplastic T lymphocytes.[36] Evidence for increased cytokine production has been shown *in vitro* for cells derived from patients prone to develop hemophagocytic syndromes. [37,38] More recent studies have implicated increased production of gamma interferon, MIP-1 alpha, and tumor necrosis factor in patients with hemophagocytic syndromes.[39,40] Thus, it has been hypothesized that in certain clinical situations, in particular in association with defective T-cell function, a state of abnormal immune regulation exists: A precipitating event such as an infection results in marked stimulation of the immune system. T cells become activated and elaborate cytokines. Cytokine production fails to be shut off, however, possibly because of abnormal feedback regulation. The excessive cytokine production continues unchecked, with marked stimulation of mononuclear phagocytes and the development of a hemophagocytic syndrome.

Additional evidence for excessive immune stimulation in the hemophagocytic syndromes is the marked elevation of serum soluble IL-2 receptor (SIL2-R) levels observed in affected patients with active disease. [41] This molecule, which is a truncated (40- to 45-kd) form of the 55-kd molecule designated CD25, is released by activated lymphocytes. Although high levels of circulating SIL2-R originally had been found in patients with human T-cell leukemia-lymphoma virus–associated T-cell leukemia or hairy cell leukemia, the highest levels of SIL2-R that have been measured occur in FEL. Because SIL2-R binds IL-2, it may interfere with normal immunoregulation in these patients. Finally, although it is tempting to hope that SIL2-R may be the elusive marker for FEL, the normal or near normal levels during clinical remission of the disease, as well as its elevation in IAHS, indicate that this is not the case. The findings do suggest, however, that the T lymphocyte could actually be the central cell in the pathogenetic process seen in the hemophagocytic syndromes and that the monocyte-macrophage proliferation is secondary.

In the case of FEL, the pathophysiology of the disease includes an element of immunodeficiency associated with plasma inhibitory activity. [42] The cell-mediated immune defects may be responsible for the opportunistic infections frequently contracted as terminal events in patients with FEL. The immune defects are secondary, however, because treatment (e.g., plasmapheresis) can remove plasma immunosuppressive activity and can result in complete recovery of cellular immunodeficiency *in vivo*.[43] How this secondary immunodeficiency relates to the underlying genetic (autosomal recessive) disease and to the unknown trigger of the clinical exacerbations of FEL, is unknown. Recently, however, a subgroup of patients with FEL has been shown to have a genetic defect in perforin, [44] a molecule critical to cytotoxic function of both T lymphocytes and natural killer cells. This defect may underlie the defective cellular cytotoxicity [42] in FEL and, in turn, the altered immunoregulation (uncontrolled cellular immune activation), although this remains to be investigated.

The physiology and pathogenesis of IAHS and FEL are similar, if not identical. In recognition of this similarity, these two entities are grouped together under the term *hemophagocytic lymphohistiocytosis*.

Finally, only in the case of neoplastic proliferation of cells of the monocyte-macrophage series (e.g., malignant histiocytosis) is the pathophysiology clearly one of a clonal proliferation of histologically malignant cells.

## PATHOLOGY

The International Histiocyte Society has proposed a system for the classification of the childhood histiocytoses. [45] The basis for classification is the relation of these lesions to normal histiocytic and reticulum cell subsets. This classification system has a pathologic basis because the ultimate diagnosis of all the childhood histiocytoses rests on the findings of pathologic examination. Although this schema does not include all proliferative histiocytic lesions of children or adults, it provides a conceptual approach to the diagnosis and classification of these disorders. The major forms of childhood histiocytosis are grouped into three classes, as presented in Table 26-3. In addition, Table 26-4 summarizes the most recently developed World Health Organization classification of the neoplastic disorders of the histiocytic cell and dendritic cell systems.[46]

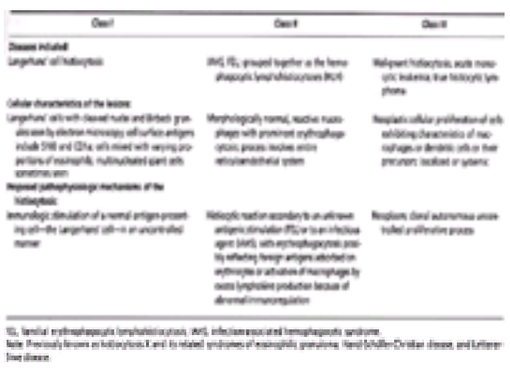


**TABLE 26-3. CLASSIFICATION OF CHILDHOOD HISTIOCYTOSES**

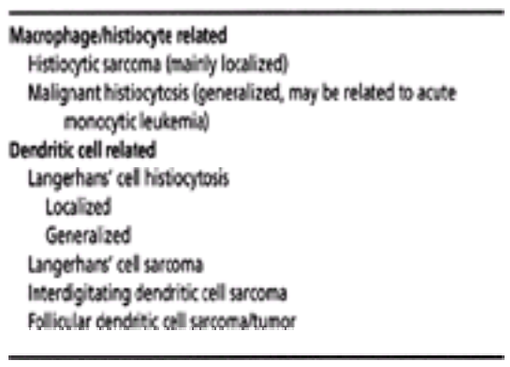



**TABLE 26-4. WORLD HEALTH ORGANIZATION CLASSIFICATION OF NEOPLASTIC DISORDERS OF HISTIOCYTES AND DENDRITIC CELLS**

### Class I Histiocytoses

Class I includes those histiocytoses in which the central cell has the histopathologic features of the Langerhans' cell. LCH, formerly known as *histiocytosis X*, is the principal disease in this class and is also the main proliferative disorder of the Langerhans' cell. LCH replaces the term *histiocytosis X* as well as the syndromes eosinophilic granuloma, Hand-Schüller-Christian disease, and Letterer-Siwe disease, which had been included under the term *histiocytosis X*.[31,47,48,49,50,51,52] and [53] LCH historically has not been considered a neoplasm but a proliferative lesion, possibly secondary to a defect in immunoregulation. [31,32] The cells of LCH demonstrate the

phenotypic characteristics of normal Langerhans' cells, including S100 positivity, CD1a (OKT6) expression, and Birbeck granules ( Fig. 26-1).[54,55 and 56] However, in contrast to normal Langerhans' cells, the cells of LCH also express leukocyte adhesion molecules, such as CD11 and CD14, typically expressed in greater density on phagocytic histiocytes (Table 26-5).[57,58] Two laboratories[32,33] have demonstrated that the lesional cells in LCH are clonal in origin. This important finding is not yet completely understood. On the one hand, it demonstrates that LCH is a proliferative process, both in patients with only single bone lesions and in those with disseminated disease. On the other hand, the distinction between reactive and malignant causes cannot be ascertained from these findings at this time. Further studies will be necessary to determine whether specific molecular genetic lesions characterize LCH cells.

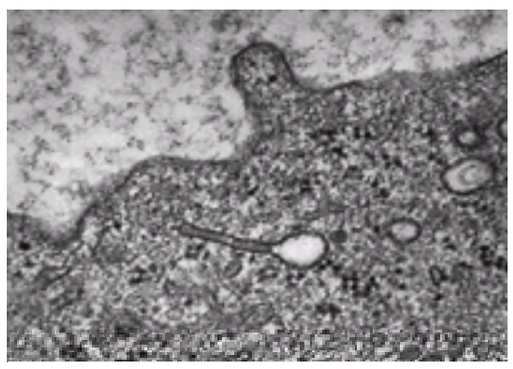


**FIGURE 26-1.** Electron micrograph of a Langerhans' cell showing Birbeck granules. This cell was obtained by needle aspiration from a patient with lymph node involvement of Langerhans' cell histiocytosis.

| Cell marker | Langerhans' cell histiocytosis | Histiocytic sarcoma | Acute monocytic leukemia | Sinus histiocytosis with massive lymphadenopathy | Follicular dendritic tumor | Anaplastic large cell lymphoma |
|---|---|---|---|---|---|---|
| CD1a | + | - | - | - | - | - |
| S100 | + | +/- | - | + | - | - |
| CD21 | - | - | - | +/- | + | - |
| CD35 | - | - | - | + | + | - |
| CD15 | - | + | + | + | - | +/- |
| CD4 | + | + | + | + | - | + |
| CD68 | - | + | + | + | - | +/- |
| CD45 (leukocyte common antigen) | + | +/- | + | + | - | +/- |
| Lysozyme | - | + | + | + | - | - |
| CD30 | - | - | - | - | - | +/- |
| ALK-1 | - | - | - | - | - | + |

+, +/-, -, indicate relative degree of expression, from strongly positive to negative.

**TABLE 26-5. PHENOTYPES OF HISTIOCYTIC (AND RELATED) PROLIFERATIVE LESIONS**

Grossly, the lesions of LCH are granulomatous and, when visible, appear yellow-brown. As such, they are easily seen in the skin. The hallmark of these lesions is the presence of Langerhans' cells by light microscopy. These cells have deeply indented nuclei and low nuclear to cytoplasmic ratios ( Fig. 26-2). The lesions in LCH may consist of either pure histiocytic infiltrates or mixed histiocytic and eosinophilic lesions, as are commonly seen in lytic bone lesions of this disease. [59,60] In addition to the varying proportion of eosinophils, phagocytic histiocytes and multinucleated giant cells are sometimes present. Necrosis may be evident. In partially involved lymph nodes, the process involves the paracortex. Although cytologic atypia may be observed in Langerhans' cells, this feature is not considered a significant prognostic factor.[61]

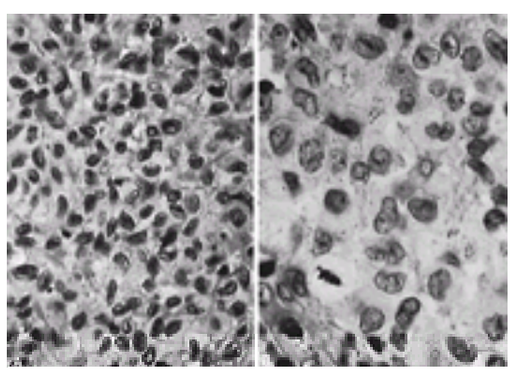


**FIGURE 26-2.** Histopathologic features of Langerhans' cell histiocytosis. **A:** Langerhans' cells exhibit delicate nuclear chromatin with fine nuclear grooves. **B:** Cytologic atypia in Langerhans' cells may be seen in some cases but are not believed to be a significant prognostic feature.

The key pathologic finding in LCH is the presence of Birbeck granules, [17] detected by electron microscopy, in cells of the lesions (Fig. 26-1). The significance of this diagnostic structure is unknown,[12] although it is also found in normal Langerhans' cells of the skin, cells whose role is to present antigens. Investigators have therefore suggested that LCH may represent an uncontrolled immunologic reaction to an unknown foreign antigen. [62] In support of this possibility are the findings of autocytotoxicity and cytokine elaboration in the lesions, previously discussed. The presence of Birbeck granules in cells of the lesions is the diagnostic finding in LCH.

Curiously, however, the cytologic atypia and particularly the Birbeck granules found by electron microscopy usually are not present in brain or liver tissue, even when these organs are clinically involved in the disease process. [63] Therefore, the lesions in these organ systems may possibly result from a different pathogenetic process.

Alternatively, the absence of Langerhans' cells in these organs may possibly prevent the characteristic expression of the granulomas of LCH. As in the analogous situation in hemophagocytic syndromes, in which a T-cell regulatory abnormality may possibly be central to the disease process, it may be that the Langerhans' cell in LCH is not central to this immunopathologic process.

As noted earlier, it has been suggested that LCH may be a malignant disease. The finding of clonality of LCH cells bears on this point, but further studies will be required to resolve this issue. Moreover, lesions with the histologic features of LCH have been described in lymph nodes in association with various malignant neoplasms, most often Hodgkin's disease.[66,67] In this situation, LCH appears to be a finding incidental to a localized process and does not have any independent prognostic importance. Finally, rare malignant proliferations with a Langerhans' cell phenotype have been described, [69,70] as discussed further in the section on class III histiocytoses.

### Class II Histiocytoses

The class II histiocytoses represent the largest group of disorders and include the nonmalignant histiocytoses in which the accumulating mononuclear cell is of the phagocytic (antigen-processing) cell type ( Table 26-1). This finding contrasts these disorders with the class I diseases, which are also reactive histiocytoses but of antigen-presenting or dendritic cell type. In the class II histiocytoses, the normal monocyte-macrophage is the predominant cell, frequently in a mixed lymphohistiocytic infiltrate. The characteristic findings on lymph node biopsy include infiltration of the nodes, especially of the sinusoids, cortex, and paracortex,

without effacement of nodal architecture. Involved lymph nodes may later show lymphocytic depletion with increased numbers of histiocytes.

The striking major histopathologic finding in the class II histiocytoses (FEL and IAHS) is the morphologically normal appearance of the involved cells; no cytologic atypia are noted (Fig. 26-3). Marked histiocytic proliferation is observed throughout the reticuloendothelial system. Sites most markedly affected include the bone marrow, splenic red pulp, hepatic sinusoids, and lymph node sinuses. [36,71]

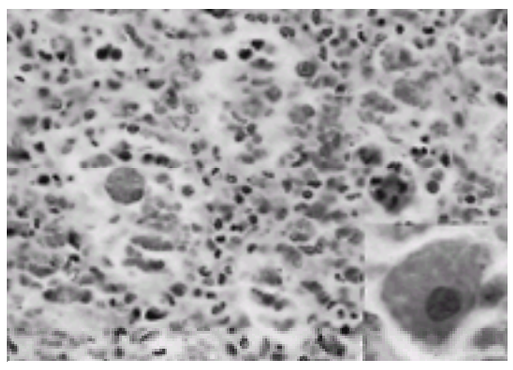


**FIGURE 26-3.** Infection-associated hemophagocytic syndrome. Histiocytic infiltrates with prominent erythrophagocytosis ( *inset*) are characteristic of both this syndrome and familial erythrophagocytic lymphohistiocytosis. Histiocytes are cytologically normal.

Involvement of the bone marrow, always obvious at the time of diagnosis of IAHS, may be delayed in FEL. In FEL, the initial marrow biopsy may show erythroid hyperplasia, without hemophagocytosis. Therefore, other sites should be biopsied to document FEL in an affected child with a positive family history. [72]

Cytologically, the histiocytes appear activated, with abundant cytoplasm and prominent phagocytosis of the formed elements of the blood, including erythrocytes, leukocytes, and platelets. These reactive histiocytes also have low nuclear to cytoplasmic ratios, mature nuclear chromatin, and inconspicuous nucleoli. Striking erythrophagocytosis and, in fact, phagocytosis of all cellular blood elements are characteristic of both IAHS and FEL. On histopathologic criteria alone, FEL and IAHS may be indistinguishable from each other but should be clearly distinguishable from both class I and class III histiocytoses. The striking similarity between FEL and IAHS suggests that one pathogenetic mechanism may be central to both diseases. This possibility remains to be elucidated.

With respect to the evolution of the lesions, resolution of the infection in the case of IAHS sometimes results in complete resolution of the histiocytic infiltration and the erythrophagocytosis. Similarly, apparent clinical remission in FEL is associated with disappearance of the pathologic infiltrates. Thus, the class II syndromes are characterized by a clearly secondary accumulation of histiocytes. The mechanisms causing these accumulations, however, remain unknown. In both cases, electron microscopy is negative for the Birbeck granules that characterize the cells of LCH, and the cellular morphology is also different from that of LCH cells. The mixed lymphohistiocytic infiltrates further help to distinguish the disseminated class II histiocytoses from class I disease, in which either mixed histiocytic and eosinophilic or pure histiocytic infiltrates are seen.

Several other rare class II histiocytoses are mentioned in the next few paragraphs, primarily because these disorders enter into the differential diagnosis of lymphadenopathy or skin lesions in which histiocytic infiltration is prominent. In sinus histiocytosis with massive lymphadenopathy (SHML), affected lymph nodes demonstrate a marked fibrous thickening of the capsule. Residual follicles are usually present and demonstrate a florid follicular hyperplasia. Consistent with the polyclonal hypergammaglobulinemia seen in these patients, a prominent plasmacytosis is present as well. The sinuses and interfollicular regions are expanded by a marked histiocytic proliferation. The histiocytes have abundant clear cytoplasm and distinct cytoplasmic membranes. A characteristic feature is the phenomenon of emperipolesis in which apparently viable lymphocytes and plasma cells are identified within vacuoles within the cytoplasm of the histiocytic cells. The nuclei of the proliferating histiocytes appear activated and may contain small but distinct nucleoli. Cytologic anaplasia indicative of malignancy has not been described, however.

Phenotypically, the cells of SHML demonstrate many of the properties of phagocytic macrophages. [66] The cells contain abundant activity of lysosomal enzymes. They also demonstrate positivity for S100 protein. Although S100 protein is more characteristic of IRCs, weak activity may be seen in normal and reactive sinus histiocytes. The cells of SHML are negative with the dendritic reticular cell antigen and CD1a monoclonal antibodies, but they demonstrate reactivity for CD4, CD11, CD14, CD25, and transferrin receptors. All of the foregoing are present on normal histiocytes and macrophages.

In histiocytic necrotizing lymphadenitis (Kikuchi's disease), histiocytes constitute a significant component of the inflammatory lesion that characterizes this disease.[73,74 and 75] The cause of this reactive lymphadenopathy is unknown. Kikuchi's disease is characterized by a focal necrotizing lesion within lymph nodes, usually located in the paracortex. Although karyorrhexis is conspicuous, neutrophils are absent. The proliferating cells are predominantly histiocytes and immunoblasts. The process is often misdiagnosed as a large cell or histiocytic lymphoma because of the prominent immunoblastic component and partial obliteration of lymph node architecture that may be seen.[67] The disease occurs commonly in young women but is rare in the pediatric age group.

Two pediatric benign histiocytic proliferative disorders are characterized primarily by skin involvement with multiple cutaneous nodules. In juvenile xanthogranuloma (multiple cutaneous nodules), lesions are composed of a monotonous cellular infiltrate of histiocytes with frequent multinucleated Touton giant cells in the subcutaneous tissue. The cytoplasm is abundant and eosinophilic. Cytologically, the cells appear benign. The cells cytochemically and ultrastructurally have the features of macrophages.[76] They are strongly positive for lysozyme.

In self-healing reticulohistiocytosis, the cutaneous nodules are composed of large histiocytic cells that may demonstrate cytologic atypia. This feature distinguishes this disease from juvenile xanthogranuloma, in which cytologic atypia are less prominent. Multinucleated forms may be present and mitotic figures are observed. The cells contain either foamy or eosinophilic cytoplasm that resembles ground glass. Erythrophagocytosis is not a conspicuous feature.

### Class III Histiocytoses

Class III comprises the malignant disorders of mononuclear phagocytes, including acute monocytic leukemia, malignant histiocytosis, and true histiocytic sarcoma. Also included in this class are the rare cases in which the malignant histiocytic cell is the Langerhans' cell. [45]

These three malignancies of mononuclear phagocytes represent a spectrum in terms of their degree of dissemination, and can be conceptually related to different stages of maturation and differentiation in the mononuclear phagocytic series. [57] Acute monocytic leukemia relates to a bone marrow–derived monoblast. This malignancy arises in the bone marrow compartment with secondary involvement of the peripheral blood and usually an elevated white blood cell count. In contrast to acute myeloid leukemia, one sees a higher incidence of involvement of nonhematopoietic sites, with frequent involvement of skin and gingiva. Hepatosplenomegaly and lymphadenopathy are common (25% and 50%, respectively).[77] Acute monocytic leukemia is discussed further in Chapter 20. Malignant histiocytosis represents a malignancy of mononuclear phagocytes that are intermediate in differentiation between monocytes and monoblasts and fixed tissue histiocytes. In many instances, the syndromes of acute monocytic leukemia and malignant histiocytosis merge, and the distinction may be arbitrary and semantic. [78,79]

Malignant histiocytosis is a systemic malignant disease involving the entire reticuloendothelial system. [80,81] Within the lymphoreticular system there is preferential involvement of sites normally populated by histiocytes, such as lymph node sinuses, splenic red pulp, and hepatic sinusoids. Bone marrow involvement is common, and although abnormal cells can be seen in the peripheral blood, if peripheral blood involvement is extensive, a diagnosis of acute monocytic leukemia should be considered. Other frequent sites of involvement include skin and bone. [82]

The cells in malignant histiocytosis have atypical characteristics ( Fig. 26-4). The nucleus is large, with a reticular chromatin pattern and prominent nucleolus. Cells exhibit a basophilic cytoplasm and stain positively for acid phosphatase and nonspecific esterase. [5,14] Erythrophagocytosis may be present but is not prominent, as in the class II histiocytoses. Even when phagocytosis is observed, it is clinically insignificant. Furthermore, on careful examination of cellular morphology, it has been observed that the phagocytosis is generally the result of reactive infiltrating cells within the tumorous lesions. Importantly, particularly in children, most neoplasms

formerly diagnosed as malignant histiocytosis represent anaplastic large cell lymphomas (ALCLs) (see below). The clinical syndrome of histiocytic medullary reticulosis, characterized by hepatosplenomegaly, pancytopenia, and jaundice, once thought to be a variant of malignant histiocytosis, is recognized now as a manifestation of the class II histiocytoses, most often IAHS.

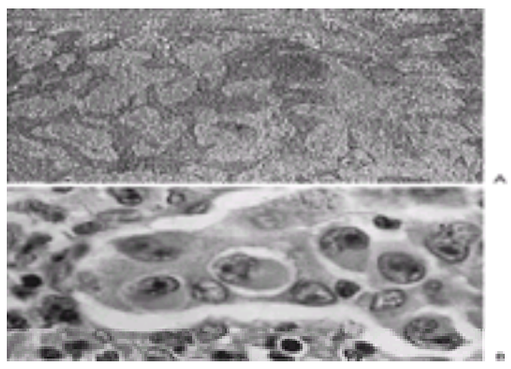


**FIGURE 26-4.** Malignant histiocytosis. Neoplastic cells **(A)** preferentially invade lymph nodes sinuses and, as seen in **(B)**, exhibit cytologically malignant features.

The end point of the spectrum is the rarest form, histiocytic sarcoma, which is a malignancy of the mononuclear phagocytic series at the stage of fixed tissue histiocytes. As such, the lesions in histiocytic sarcoma are localized and discrete. In addition to the reticuloendothelial system, other sites of involvement include skin, gastrointestinal tract, and bone.[83] Cytologically, the cells are unquestionably malignant. Without the benefit of specialized diagnostic tools, these lesions would fall into the category of large cell, immunoblastic lymphomas in the working formulation.[84]

Enzyme cytochemistry and histochemistry remain a reliable adjunct to morphology in the diagnosis of malignant diseases of the mononuclear phagocytic system. The cells have diffuse staining for nonspecific esterase activity, which is usually at least partially fluoride sensitive.[85] Preferable methods for detection of esterase activity include the a-naphthyl butyrate esterase reaction because activity is not observed in myeloid cells.[20] Myeloid cells also contain minimal, if any, activity for a-naphthyl acetate esterase. The naphthol ASD acetate esterase reaction requires the use of fluoride to distinguish myeloid and mononuclear phagocytic cells. Activity for acid phosphatase and b-glucuronidase is usually present as well. In all cases, the activity should be diffuse throughout the cytoplasm and not punctate. Punctate reactivity localized to the Golgi region is more characteristic of lymphoid than of mononuclear phagocytic cells. Caution should be exercised in the use of enzyme cytochemistry because these enzymes are not specific for mononuclear phagocytes and can be seen in certain lymphomas, carcinomas, and sarcomas.[86]

Most malignancies previously diagnosed as malignant histiocytosis in children actually represent ALCLs.[87,88] Indeed, most instances of so-called malignant histiocytosis appear to represent this variant of Ki-1 (CD30)-positive lymphoma. In this tumor, the malignant cells have a propensity to invade lymphoid sinuses. Because of the sinusoidal location of the tumor cells, misdiagnosis as malignant histiocytosis or metastatic carcinoma has been common.[87] In most cases studied, the malignant cells express some T-cell antigens, although the cells have an aberrant phenotype. T-cell gene rearrangement has also been shown in some instances.[89,90] and[91] A consistent feature is the expression of the Hodgkin's disease–associated antigen CD30, detected by Ki-1 and BerH2. This antigen, although present on the malignant cells of Hodgkin's disease, is also found in activated T and B lymphocytes (but not in macrophages).

ALCL has been associated with a common recurring cytogenetic translocation.[92,93] This translocation, t(2;5)(p23;q35), was also described previously in cell lines derived from cases of so-called malignant histiocytosis[94] that in retrospect, were derived from ALCL. The translocation involves the anaplastic lymphoma kinase (ALK) tyrosine kinase gene on chromosome 2, and the nucleophosmin (NPM) gene on chromosome 5. More recently, variant translocations have been identified involving the ALK gene and partners other than NPM. ALK tyrosine kinase is overexpressed in the malignant cells, and now can readily be detected in routine paraffin sections with the ALK-1 monoclonal antibody.[95]

ALCL can occur in all age groups, but it appears to be common in children and young adults.[80] A high incidence of cutaneous disease has been reported. The skin lesions are deep dermal, but ulceration of the overlying epidermis may be seen in larger tumors. The process should be approached clinically as a large cell or aggressive non-Hodgkin's lymphoma. A histiocyte-rich variant has also been described in children and must be distinguished from both class II and III histiocytoses (discussed in Chapter 24).[96]

To summarize, the pathologic classification of the childhood histiocytoses includes LCH in class I, the class II histiocytoses that result from the accumulation of benign histiocytes as a secondary response to disease processes of unknown pathogenic mechanisms, and the class III malignant histiocytic disorders. Histopathologic diagnostic criteria are summarized in Table 26-1. Accurate diagnosis of the childhood histiocytoses is vital. The presence of those pathologic features of definitive value in classifying these disorders must be identified: Birbeck granules uniformly and only in class I histiocytoses; malignant characteristics uniformly and only in class III histiocytoses; and benign reactive histiocytes comprising class II histiocytoses. Attempting to make a clinical diagnosis without these histopathologic findings is inappropriate.

## CLINICAL PRESENTATION AND TREATMENT

### Class I Histiocytoses

#### *Langerhans' Cell Histiocytosis*

The clinical presentation of LCH varies from mild discomfort and irritability to specific lytic bone lesions (Fig. 26-5) that may have been diagnosed only incidentally on a radiograph obtained for another reason.[97,98,99] and[100] Other symptoms may include chronic otitis, diabetes insipidus, or generalized symptoms such as fever and weight loss. This varied clinical presentation may make the diagnosis of LCH difficult. As noted previously, the definitive diagnosis (Table 26-6) rests on confirmation of the presence of the Birbeck granule–positive cells in the lesions of the disease as determined by electron microscopy, or CD1a-positive lesional cells. Other findings include positive immunostaining for S100 protein, which is not present on normal histiocytes.[11] The biopsy sample must be obtained from clearly involved tissue because normal Langerhans' cells containing Birbeck granules are present in the skin as well. Multiple organ systems may be involved in this disorder (Table 26-7).

**FIGURE 26-5.** Langerhans' cell histiocytosis. Radiograph of the characteristic lytic bone lesion, as seen in the skull of a patient whose other findings included exophthalmos and diabetes insipidus.

| Class I (Langerhans' cell histiocytosis) | Class II (infection-associated hemophagocytic syndrome) | Class II (familial erythrophagocytic lymphohistiocytosis) | Class III |
|---|---|---|---|
| **Clinical presentation** | | | |
| Wide spectrum, from mild discomfort related to lesions (lytic bone lesions, chronic otitis, diabetes insipidus) to generalized symptoms, including fever and weight loss | Pancytopenia, hepatosplenomegaly, fever, coagulopathy | Irritability, fever, vomiting, sometimes with a coagulopathy and hepatosplenomegaly | Variable, from systemic disease to acute monocytic leukemia to localized mass in true histiocytic lymphoma |
| **Diagnostic findings** | | | |
| Intact granules and CD1a expression in lesional cells | Morphologically normal macrophages, often showing related infection and negative family history | Morphologically normal bone phagocytes and macrophages, lack of specific infectious etiology, sometimes positive family history | Malignant macrophages or dendritic cells |
| **Prognosis** | | | |
| Variable; may be self-resolving disease process in some cases | Possible resolution if underlying infection is controlled and immunosuppression can be reversed | Extremely poor; uniformly rapidly fatal | Poor for acute monocytic leukemia; improving for MH and HT, up to 75% survival at 4% reported with appropriate therapy |
| **Recommended treatment** | | | |
| None to mild (radiation or chemotherapy limited to one or a few sites), and steroids for certain lesions | Avoidance of immunosuppressive therapy; experimental: etoposide | Experimental: allogeneic bone marrow transplantation | Doxorubicin (Adriamycin) in a combination chemotherapy regimen for MH and HT; appropriate treatment for acute monocytic leukemia |

HT, histiocytic sarcoma; MH, malignant histiocytosis.

**TABLE 26-6. CLINICAL, PROGNOSTIC, AND THERAPEUTIC ASPECTS OF THE MAJOR CHILDHOOD HISTIOCYTOSES**

| Site | Percentage of cases involved (%) |
|---|---|
| Bone | 80 |
| Skin | 60 |
| Liver, spleen, lymph nodes | 33 |
| Bone marrow | 30 |
| Lungs | 25 |
| Orbit | 25 |
| Orodental | 20 |
| Otological | 20 |
| Central nervous system | |
| Diabetes insipidus | 15 |
| Hydrocephalus, nerve palsies | <5 |
| Gastrointestinal tract | <5 |

From Callihan TR. The surgical pathology of the differentiated histiocytoses. In Jaffe ES, ed. Surgical pathology of the lymph nodes and related organs. Philadelphia: WB Saunders, 1985:357, with permission.

**TABLE 26-7. ORGAN SYSTEM INVOLVEMENT IN LANGERHANS' CELL HISTIOCYTOSIS**

The clinical hallmark of LCH has been the presence of lytic bone lesions ( Fig. 26-5),[98,101] but clinical involvement may be variable. Formerly, when either single or multiple bone lesions alone were present, the disease was referred to as *eosinophilic granuloma*.[53] When granulomas were more widespread, causing bone lesions, diabetes insipidus (by involvement of the pituitary), and exophthalmos (by the presence of retro-orbital granulomas), the disease was termed *Hand-Schüller-Christian disease*.[48,50] Finally, the disseminated form of LCH was previously called *Letterer-Siwe disease*.[31,51] This last presentation is more commonly seen in infants and in children younger than 2 years and is characterized by wasting, hepatosplenomegaly, generalized lymphadenopathy, anemia, and sometimes pancytopenia. The milder forms of LCH are seen primarily in older children and less commonly in adults. The clinical finding of seborrheic dermatitis deserves special mention. It is often present on the scalp and in infants may easily be confused with a nonspecific dermatitis. As with the other lesions of LCH, however, biopsy and examination by electron microscopy, immunohistochemistry, or both, are diagnostic. In addition to the foregoing clinical findings, virtually every tissue may be involved in LCH, including the lung and gastrointestinal tract. The manifestations of LCH may be protean. No evidence indicates, however, that a given lesion causes another lesion when no physical contact exists. Rather, both in location and in time, the individual lesions of LCH should be considered separate from each other. This consideration has implications for treatment approaches and suggests that clinical classification of the forms of LCH remains to be developed further. A comprehensive approach to the clinical and laboratory evaluation of children with LCH has been suggested by the Histiocyte Society. [102]

Central nervous system (CNS) involvement in LCH has also become increasingly recognized. A long-understood complication of LCH is the development of diabetes insipidus because of hypothalamic and pituitary histiocytic involvement. Less well known are the subtle neurologic defects that may be progressive and may develop years after the initial presentation of LCH. [63] They include hyporeflexia, spastic dysplasia, ataxia, vertigo, dysarthria, nystagmus, tremor, psychomotor retardation, and neuropsychological deficits.[64] The pathogenesis of this aspect of LCH remains enigmatic because characteristic histopathologic features are generally lacking. Understanding of CNS disease has advanced in the last several years, [63] particularly by the use of magnetic resonance imaging. This technique has enabled identification of cerebellar lesions in patients with LCH. The lesions may be mass lesions, but frequently, in the cerebellum, they are only hypodense lesions consistent with demyelination and gliosis of periventricular white matter. [65] These findings have been confirmed by biopsy. The characteristic biopsy findings of LCH, which are Langerhans' cells with Birbeck granules, are absent in these hypodense lesions. These cerebellar pathologic findings parallel the aforementioned symptoms, including ataxia and dysarthria. Clearly distinct from the well-known diabetes insipidus associated with LCH, diffuse CNS disease presents a new challenge in LCH. At the present time, no specific recommendation regarding treatment for this syndrome can be given.

The outcome of LCH varies, but in most cases the disease is a self-resolving process. The two prognostic factors appear to be age at the time of diagnosis and degree of organ involvement.[97,100] Children younger than 2 years at the time of diagnosis have a higher mortality rate than do older children. Likewise, the presence of organ dysfunction (e.g., liver, lungs, and bone marrow) is a poor prognostic sign, with liver involvement at the time of diagnosis indicating a particularly poor prognosis.[103,104] Involvement of multiple organ systems has an additive negative effect on survival. Fortunately, children with these poor prognostic features constitute fewer than 15% of LCH cases. Other than these two factors and the knowledge that the disease usually resolves completely and spontaneously, little is known about the natural evolution of the disease process and the effects of intervention.

The approaches to treatment of LCH over the last century have been as varied as the clinical presentation of the disease. [31,105,106,107,108,109] and [110] Thus, treatment has ranged from antimicrobial therapy, based on the assumption that LCH is an infectious disease, to chemotherapy, based on the belief that this disorder is an aggressive malignancy. Although some reports in the literature had supported a role for chemotherapy by demonstrating improvement in the overall survival rate compared with historical controls, careful diagnosis and stratification for severity of disease were not always applied in these studies. When such analyses were performed on a large number of patients, all uniformly classified, it appeared that disseminated LCH, with multiple-organ involvement, had a poor prognosis that had not been changed substantially by various treatments. [62] In contrast, mild disease has an excellent prognosis that also has not been changed by the administration of any particular therapy. [62,111] These findings suggested that little progress has been made in the development of definitive therapy for LCH. Because this concept is now accepted by pediatric oncologists, current treatment approaches to mild LCH tend to use less intensive therapy, when treatment is indicated. Specifically, this includes use of vinblastine or etoposide, [112] with or without the addition of prednisone, and low-dose radiation therapy. Moreover, long-term steroid therapy should be avoided because of its secondary side effects.

In the absence of a specific, definitive treatment, the therapeutic approach to the child with LCH should be directed toward preventing irreversible damage to normal tissues (permanent consequences) by LCH lesions that eventually spontaneously resolve. Thus, treatment should be directed toward halting or reversing the progression of lesions. This approach has been widely applied to cases of isolated bone involvement in LCH and may also be applicable to more disseminated disease. Even the use of mild therapy remains controversial, however, because of the absence of definitive prospective studies evaluating various treatment approaches to this entity.

Encouraging data about the value of chemotherapy in multisystem LCH came from a large nonrandomized cooperative study of LCH. [104] Patients were stratified according to risk and received mild initial chemotherapy (vinblastine, etoposide, and prednisone) and “maintenance therapy” with the same drugs as well as 6-mercaptopurine and methotrexate. The speed of resolution was rapid (median, 4 months), and the frequency of recurrence after initial resolution was low, ranging from 12% in patients with multifocal bone disease to 42% in patients with organ dysfunction. Overall, 77% of patients have remained free of disease recurrence. Permanent consequences developed after diagnosis in 20% of the patients. A striking finding of this study was the low incidence of sequelae of LCH, especially diabetes insipidus (only 10% after the initiation of treatment). [104] This finding is in contrast to the reported higher incidence of 23% (4% at diagnosis; 19% developing later).[113] The much lower incidence of late development of diabetes insipidus in the former study may reflect the early treatment of all patients, which may therefore be

valuable in reducing the complications and sequelae of LCH, even if overall mortality is not reduced.

To optimize therapy, a sufficiently large, prospective, randomized treatment study is necessary. For this reason, an international randomized trial in LCH was initiated by the Histiocyte Society in 1991. This study (LCH-1) compared etoposide and vinblastine in the treatment of disseminated LCH. Data on the risk of etoposide-associated (therapy-induced) malignancy in the setting of histiocytosis have been reviewed, and the available evidence leads to the recommendation that the study of etoposide in LCH should be continued.[114] LCH-1, now completed, showed vinblastine and etoposide to be equivalent in all respects—response, toxicity, probability of survival, and probability of disease reactivation and development of permanent consequences. The study also identified lack of rapid (within 6 weeks) response as a new prognostic indicator, predicting a high (66%) mortality rate in children with risk organ involvement (liver, lung, hematopoietic system, or spleen).[115] A relatively high rate of disease reactivation suggests that more intensive therapy may be of value for some patients. This is currently being studied, also in a randomized trial (LCH-2) by the Histiocyte Society. In tandem with systematic therapy the use of low-dose radiation therapy to specific lesions that threaten permanent damage is recommended. Doses in the range of 500 to 800 cGy are recommended for such single lesions. Systemic therapy, vinblastine (0.4 mg per kg weekly) or etoposide, with or without corticosteroids, has been used in the case of more widespread disease.

Significant numbers of children, particularly infants with multisystem disease, are “unresponsive” to mild therapy. New treatment approaches should be considered in this group of patients.[105] One such approach is the use of cyclosporine in the treatment of LCH. Several patients treated with cyclosporine had a rapid clinical response.[116] Recrudescence of the disease was observed when the drug was discontinued, however. Investigators have speculated that cyclosporine may act to reduce cytokine production,[117] thereby implicating massive cytokine production as a factor in the pathogenesis of LCH. The eventual value of cyclosporine in the treatment of LCH may lie in its use in combination with mild chemotherapy. Other experimental treatment approaches include bone marrow transplantation, which is considered for severe, unresponsive cases.

The most promising new therapeutic agent for LCH appears to be the antimetabolite 2-chlorodeoxyadenosine (2-CdA), which in addition to being lymphocytotoxic evidences potent cytotoxicity against monocytes.[118,119] 2-CdA has been effective particularly in adult patients with chronic LCH,[119] as well as in some children with refractory or recurrent LCH.[120] Further controlled studies of this agent are therefore warranted and are in progress. Finally, monoclonal antibody therapy, using CD1a as the target epitope, is also being investigated.[121] The effectiveness of these agents remains to be established.

### *Dendritic and Interdigitating Reticulum Cell Tumors*

Although LCH is the major form of histiocytosis included in class I, at least two additional rare lesions are described that have a proposed derivation from FDC and IRC, respectively.[122,123 and 124] FDC sarcomas tend to present as localized lymph node disease. Although local recurrence may occur, systemic spread does not. By contrast, IRC tumors have a more aggressive clinical course and are more appropriately included among the class III histiocytoses—that is, malignancies of histiocytic cells.

## Class II Histiocytoses

Diseases falling into the class II histiocytoses category consist of those in which reactive cells of the mononuclear phagocytic cell series, excluding Langerhans' cells, are found in the lesions. The histiocytic reaction is presumed secondary to a primary underlying disease process. Because the histiocytic proliferation may result from different pathogenetic mechanisms, one can predict that both the prognosis and treatment of the individual diseases in this class would be widely different. This is in fact the case. The two major diseases in this category are FEL and IAHS, grouped together under the common term *hemophagocytic lymphohistiocytosis*, to reflect awareness that the pathogenesis of these two diseases is similar, even though the causes are different.

### *Familial Erythrophagocytic Lymphohistiocytosis*

FEL is characterized by the presence of hemophagocytosis and a positive family history.[125,130] Early in the disease, biopsy of lymph nodes demonstrates lymphoid proliferation. Later, however, lymphoid depletion occurs. Patients exhibit multiple defects in cellular and humoral immunity; a plasma inhibitor of lymphocyte blastogenesis has been demonstrated.[42]

FEL is a rare and almost always rapidly fatal disease. An autosomal recessive pattern of inheritance has been recognized since the initial studies of Farquhar and Claireaux.[126] These findings have led to exhaustive searches for a genetic lesion. Recently, several specific chromosomal abnormalities have been documented in FEL. These include linkage to 9q21.3-22, 10q21-22, and possibly other loci.[127,128 and 129] The heterogeneity of these abnormalities suggests either that FEL is not a single disease or that the abnormalities are secondary. In favor of the former possibility is the recent association of a defect in perforin (the gene for which is mapped to 10q22) with homozygous nonsense or missense mutations in eight 10q21-22–linked FEL patients.[44]

The pathogenesis of FEL is not understood. The clinical presentation of FEL is that of a generalized disease. Affected children, usually younger than 3 or 4 years of age at the time of diagnosis, most often present with weight loss and a fever of unknown origin. They may have the overall clinical appearance of failure to thrive. Physical examination may reveal hepatosplenomegaly and sometimes a maculopapular skin eruption. This eruption is distinguished from the yellow-brown eruption of LCH by its color, which is frequently red to purple. The CNS may be involved, with symptoms including disorientation, seizures, and sometimes coma.[131] Laboratory findings may include marked hyperlipidemia, hypofibrinogenemia, and the cellular immunologic dysfunction mentioned earlier. Although investigators initially thought that some or all of these laboratory findings were related to the underlying primary disease process, this is apparently not the case, because the onset of temporary clinical remission results in normalization of all laboratory parameters.[43] It now appears more likely that these abnormalities are a secondary phenomenon, as is the histiocytic reaction, in this genetic disease of undefined pathogenesis. Chromosomal abnormalities of a nonspecific nature have been reported.[132] Erythrophagocytosis in FEL is marked, particularly late in the course of the disease, and the terminal phase, which resembles the hemophagocytic syndromes, is frequently characterized by pancytopenia and jaundice.[133]

The diagnosis of FEL rests on identification of a lymphohistiocytic infiltrate, often with conspicuous erythrophagocytosis, together with an appropriate clinical and family history. The diagnosis may be made from examination of lymph nodes, spleen, liver, bone marrow, or lungs. The presence of a positive family history is obviously helpful. Because the disease is inherited in an autosomal recessive fashion, however, this history is frequently unavailable. Often, the correct diagnosis is not reached until a second family member is affected. For a review of diagnostic criteria, the reader is referred to the Histiocyte Society's diagnostic criteria and suggested evaluation for the hemophagocytic lymphohistiocytoses (FEL and IAHS) (Table 26-8).[72]

| Required for diagnosis* | Consistent with diagnosis |
|---|---|
| **Clinical** | **Clinical** |
| Fever | Jaundice |
| Splenomegaly | Edema |
| | Lymphadenopathy |
| **Laboratory** | **Laboratory** |
| Cytopenias (affecting ≥2 of 3 lineages in the peripheral blood and not caused by a hypocellular or dysplastic bone marrow): | ↑Circulating soluble interleukin-2 receptors |
| Hemoglobin (<9.0 g/dL) | Hyperferritinemia |
| Platelets (<100 × 10⁹/L) | Cerebrospinal fluid pleocytosis (mononuclear cells) |
| Neutrophils (<1.0 × 10⁹/L) | Hepatic enzyme abnormalities |
| Hypertriglyceridemia or hypofibrinogenemia (fasting triglycerides ≥2.0 mmol/L or ≥3 SD of the normal value for age; fibrinogen ≤1.5 g/L or ≤3 SD) | ↑ Very-low-density lipoprotein<br>↓ High-density lipoprotein<br>↓ Natural killer cell activity |
| Histopathologic criteria: Hemophagocytosis in bone marrow or spleen or lymph nodes; no evidence of malignancy | |

*All are required for the diagnosis of hemophagocytic lymphohistiocytosis. [Infection-associated hemophagocytic syndrome or familial erythrophagocytic lymphohistiocytosis (FEL)]; in addition, the diagnosis of FEL is justified by a positive family history and parental consanguinity is suggestive.
Adapted from Henter J-I, Elinder G, Ost A. Diagnostic guidelines for hemophagocytic lymphohistiocytosis. Semin Oncol 1991;18:29.

**TABLE 26-8. CLINICAL AND LABORATORY FINDINGS IN THE HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSES**

The rapid and fulminant course of FEL, coupled with findings of marked lymphohistiocytic organ infiltration, has resulted in the erroneous consideration of this disease as malignant. Consequently, cytotoxic chemotherapy has been used in the treatment of FEL.[134] Agents that have been used include vinblastine, etoposide, and other more convenient antileukemic drugs. In general, however, treatment has been unsuccessful, with the usual rapidly fatal course of the disease only slightly prolonged

by the administration of therapy. The lack of definitive benefit from chemotherapy is not surprising given that the disease is histopathologically benign. The extent to which inhibition of the hemophagocytic process may ameliorate the pathogenesis or the evolution of FEL remains to be determined, although clinical improvement is universally associated with quiescence of the hemophagocytic process. Because of the relative lack of success of these treatments, other, more experimental therapeutic approaches have been used. Plasma exchange therapy, consisting of a series of plasmaphereses or plasma exchange transfusions, was based on the findings of circulating immunosuppressive activity in this disease. [43] After exchange therapy, reduction in plasma inhibitory activity and reversal of the depressed cellular immune responses have been observed. Although clinical improvement was noted in an early trial of this approach, the disease subsequently relapsed.

The most promising treatment of FEL appears to be bone marrow transplantation. This approach is based on the hypothesis that the disease represents either an autonomous or uncontrolled proliferation of lymphocytes and histiocytes. Allogeneic bone marrow transplantation may be successful in curing some patients with FEL. According to results of a recent comprehensive study of 122 patients, allogeneic bone marrow transplantation resulted in a 66% estimated 5-year survival versus only 10% for patients treated with chemotherapy.[135] Consequently, a controlled study of chemotherapy and allogeneic bone marrow transplantation for FEL is currently in progress.[136] Although further understanding of the disease process is essential to identify the most effective treatment for FEL, clearly the transplantation results are encouraging.

### *Infection-Associated Hemophagocytic Syndrome*

IAHS was first described as a response to a viral infection in an immunocompromised host.[71] Subsequently IAHS has been reported in association with various infections, including viral, bacterial, fungal, and parasitic infections. [137,138] This hemophagocytic syndrome usually occurs in a setting of immunodeficiency. The immunodeficiency may be iatrogenic, as in organ transplant recipients receiving immunosuppression, or congenital. IAHS has also been seen in association with lymphoid malignant diseases, most often of the T-cell type, such as acute lymphocytic leukemia and the angiocentric immunoproliferative disorders. [36,139,140] and [141] The clinical features of IAHS are similar to those of FEL and include fever and other constitutional symptoms, liver function and coagulation abnormalities, and anemia. The coagulopathy appears to be multifactorial. Coagulation factor levels are depressed, presumably secondary to liver disease, and a component of consumption coagulopathy is also seen.[71] Although the overwhelming nature of the histiocytic proliferation has resulted in confusion of this syndrome with malignant histiocytosis, histopathologic analysis can distinguish these two forms of childhood histiocytosis. Moreover, the clinical features associated with IAHS are not characteristic of the true histiocytic malignancies, malignant histiocytosis and histiocytic sarcoma. [57] One must distinguish the benign IAHS from a true histiocytic malignancy.

The diagnosis of IAHS is most readily made on bone marrow aspirate, in which benign-appearing histiocytes containing platelets and red blood cells are frequently seen. Lymph nodes usually demonstrate lymphoid depletion and marked infiltration by benign histiocytes. Because the histiocytes are normal and reactive, they demonstrate all the phenotypic and enzyme cytochemical characteristics of normal activated macrophages.[36]

As mentioned previously, IAHS usually occurs in a clinical setting characterized by immunodeficiency. If the diagnosis is made, immunosuppression should be withdrawn and supportive care instituted. Treatment for the underlying infection should be instituted. Acyclovir administration has been useful in some patients with Epstein-Barr viral infections and IAHS.[142] Cytotoxic chemotherapy is generally contraindicated for IAHS, although etoposide or another drug that interrupts the pathogenesis of IAHS may be beneficial in reducing the expression of the syndrome while the underlying process (infectious) resolves. Chemotherapy has been found to be very effective in treating EBV-related hemophagocytic lymphohistiocytosis (IAHS) in Japan. [143]

Currently, caution is still suggested in the use of etoposide, both for LCH and for the class II histiocytoses, because of the reported risk of secondary myeloid leukemia in patients treated with the agent (see Chapter 20).[144] Because the secondary leukemias were found in patients with primary malignancies treated with intensive combination chemotherapy (in contrast to use of etoposide as a single agent in the treatment of histiocytosis), whether this drug should be avoided in the histiocytoses, in which etoposide has been found to be a particularly active agent, remains an issue. Evidence available to date [114,115] suggests that the risk of secondary myeloid leukemia in this particular group of patients (those with histiocytosis) is low (on the order of 1%), and therefore use of the agent should be continued, especially given that the mortality rate of the patients treated may be high. Attention to this issue has resulted in the interesting findings of a high association between LCH and malignancies in patients with LCH, with either disease (malignancy or LCH) preceding the other such that a causal relationship between the two cannot be established at this time.[68]

### *Other Class II Histiocytoses*

SHML (Rosai-Dorfman disease) presents primarily in the first two decades of life.[145] It affects boys and girls equally and is seen more often in blacks than in other races. Patients generally present with massive cervical lymphadenopathy, although many other sites can be involved, including skin, orbit, bone, salivary gland, and upper respiratory tract. Patients usually exhibit some systemic symptoms, including fever, increased erythrocyte sedimentation rate, polyclonal hypergammaglobulinemia, and neutrophilic leukocytosis.[146] The etiology of SHML remains obscure. A relation to an underlying immunodeficiency has been postulated. Although SHML is not considered a malignant disorder, considerable morbidity and mortality can result. [147] The lesions are locally destructive and can involve almost every organ system. Both radiation and chemotherapy have been used with some success, and in some cases the lesions spontaneously regress. [148,149]

Histiocytic necrotizing lymphadenitis is a reactive lymphadenopathy, the cause of which is unknown. The disorder usually presents in cervical or axillary lymph nodes. It affects women much more often than men and is more common in Asians than in whites or blacks. The peak incidence is in the third decade of life, but it may be seen in children and adolescents. Clinically, patients exhibit mild constitutional symptoms, fever, fatigue, and localized adenopathy. An infectious etiology is suspected but has not been confirmed. No bacteria or fungi are seen on special stains, and serologic evaluations for toxoplasmosis or Epstein-Barr viral infection have been negative. Because this disorder is entirely benign and has a self-limited clinical course, correct diagnosis is critical. No treatment is indicated.

Juvenile xanthogranuloma is a benign histiocytic lesion of the skin first described by Helwig and Hackney. [150] It occurs predominantly in neonates and young children. The process is characterized by one or more cutaneous nodules, often restricted to the head, neck, trunk, and proximal portions of the extremities. Extracutaneous involvement has also been reported. The lesions usually persist for 1 to 2 years and then spontaneously resolve. [150]

Self-healing reticulohistiocytosis presents in the perineonatal period. [151] Patients have multiple firm cutaneous nodules that range from dark red to dark blue. The nodules resolve spontaneously within 2 or 3 months. In some patients, hematologic abnormalities such as neutropenia, lymphocytosis, or both, have been reported. The process is distinguished from juvenile xanthogranuloma in that more cytologic atypia of the histiocytic cells is evident. The clinical course is benign, and no therapy other than supportive care is indicated.

### Class III Histiocytoses

The histiocytoses that are true neoplasms constitute a minority of the childhood histiocytoses. As noted previously, three major forms are known (acute monocytic leukemia, malignant histiocytosis, and true histiocytic sarcoma). Acute monocytic leukemia, included in this class because of its cell origin, is discussed in Chapter 20.

True malignant histiocytosis is a rare disease. When it occurs, it is usually seen in older children and adults, but it has been described in young children. Affected children present with symptoms of a generalized illness. Clinical symptoms and findings include fever, wasting, lymphadenopathy, and hepatosplenomegaly. Raised skin lesions, peripheral lymphadenopathy, and subcutaneous inflammatory infiltrates also may be seen. Each of these findings represents infiltration by tumor cells. Thus, the clinical presentation may be similar to that of FEL or IAHS. Indeed, some cases diagnosed as malignant histiocytosis in the past may have actually been one of the hemophagocytic syndromes in class II.[152,153]

As discussed earlier in the section on pathology, the diagnosis is best made by lymph node biopsy. Circulating tumor cells are rarely observed in these patients, who nevertheless almost always exhibit some degree of peripheral pancytopenia. Malignant histiocytosis must be distinguished from the other histiocytoses and from disseminated (stage IV) Hodgkin's disease, immunoblastic non-Hodgkin's lymphoma, and immunoblastic lymphadenopathy. In these latter disorders, immunophenotypic characterization may be a helpful diagnostic tool.

True histiocytic sarcomas may involve lymph nodes, the reticuloendothelial system, and skin and bone. When initially confined to the skin, this disease has been reported to pursue an indolent clinical course, with spontaneous regression of lesions observed in some cases. [154] Lesions previously thought to be of histiocytic derivation, such as regressing atypical histiocytosis, [155,156] are now known to be of T-cell derivation, however, and are part of the spectrum of CD30-positive lymphoproliferative disorders of the skin—that is, lymphomatoid papulosis and cutaneous ALCL. Therefore, earlier clinical series of cutaneous histiocytoses must be

reevaluated in light of newer information.

The appropriate treatment of the class III malignant histiocytic disorders of childhood is based on accurate diagnosis. Acute monocytic leukemia should be approached with treatment appropriate for that disorder. Because the diagnosis and classification of malignant histiocytic disorders have undergone such dramatic change in recent years, critical evaluation of previous clinical series is problematic, and conclusions regarding therapeutic efficacy are difficult. As noted earlier, many cases previously diagnosed as malignant histiocytosis represented either hemophagocytic syndromes (a benign disorder) or ALCL (an aggressive non-Hodgkin's lymphoma) (see Chapter 24).[57,89,157,158]

Malignant histiocytosis is one childhood histiocytosis in which treatment has clearly changed the prognosis. Formerly, this disease was considered to be almost uniformly fatal. Survival has now improved, however, apparently because of the addition of doxorubicin (Adriamycin) to the combination chemotherapy treatment regimens for malignant histiocytosis.[62] With the addition of this agent, a median survival of less than 6 months has been increased to one of approximately 40 months. Investigators have recommended that children with malignant histiocytosis receive induction therapy with vincristine, prednisone, cyclophosphamide, and doxorubicin, and subsequent maintenance therapy consisting of vincristine, cyclophosphamide, and doxorubicin.[81] The use of intensive, aggressive, doxorubicin-containing regimens also appears to be effective in the treatment of histiocytic sarcomas.[57]

## CONCLUSION

Most of the childhood histiocytoses have historically been discussed in pediatric oncology textbooks because of their traditional assignment to this subspecialty of pediatrics. Because most of these disorders are not true malignant diseases, however, the use of antineoplastic agents in their treatment should be carefully limited (with the exception of the treatment of class III histiocytoses). Careful documentation of the natural history of the childhood histiocytoses, use of the new system of pathologic classification discussed in this chapter, and careful and complete clinical and laboratory evaluation may help to provide a framework for the development of optimal therapeutic approaches. Ultimately, improvement in the outcome for children with the histiocytoses will benefit from further elucidation of the etiology and pathogenesis of these diseases.

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# 27

# TUMORS OF THE CENTRAL NERVOUS SYSTEM

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## INTRODUCTION

Tumors of the central nervous system (CNS) on the whole represent the second most common pediatric cancer diagnosed in the United States each year. Depending on the upper age chosen, the number of children, adolescents, and young adults who received diagnoses of a CNS tumor in 1999 ranged between 1,700 (for ages 0 to 14 years) and 2,200 (for ages 0 to 20 years).[1] The numbers are higher if one includes such “benign” diagnoses as craniopharyngioma and choroid plexus papilloma (CPPs).[2,3] Figure 27-1 shows the approximate incidence of the common pediatric CNS tumors.

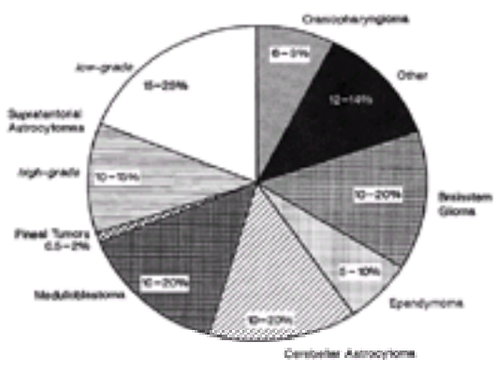



**FIGURE 27-1.** Approximate incidence of common central nervous system tumors in children.

Experience suggests that the morbidity from disease and therapy is significant in terms of physical and intellectual sequelae and, although not quantitated, likely exceeds that associated with other pediatric malignancies. Deaths caused by CNS tumors are the highest among pediatric cancers.[4] Although modest increases in cure rates have been realized, children with a CNS tumor remain a challenge for those who care for them. The authorship of this chapter reflects the multidisciplinary approach required to meet the challenge of increasing cure rates for children with brain tumors. The disciplines represented are by no means comprehensive, however. Optimal care of children with brain tumors involves the contribution of nurses; psychologists; physical, occupational, and speech therapists; and nutritional experts as well. The chapter reviews our understanding of the biology of brain tumors, the principles associated with each of the diagnostic and treatment modalities, management of the more frequently encountered tumors, and long-term effects of both the diseases and their treatment.

## EPIDEMIOLOGY

In the early 1990s, the incidence of CNS tumors in children appeared to be on the increase. Whereas the incidence during the years 1977 to 1981 was 2.7 cases per 100,000 children, from 1990 to 1994, the incidence was 3.3 cases per 100,000 children.[5] Although not definitively proven so, this higher incidence was likely due to the increased use of magnetic resonance imaging (MRI) to evaluate children with neurologic conditions and to an increase in microscopical confirmation of brainstem lesions.[6] Through these changes, the number of brainstem gliomas and supratentorial tumors that were identified increased, primarily as low-grade astrocytomas (LGAs). Other factors also may have contributed to the apparent increased incidence of pediatric brain tumors. Stereotactic biopsies were used more to document histologies of tumors at nonbrainstem sites; in the past, these tumors would not have undergone biopsy. The World Health Organization (WHO) classification of malignant gliomas also changed during this time, resulting in a shift of some diagnoses from benign to malignant. These combined factors affected the detection and reporting of brain tumors.[6] Conversely, ascertaining that the increase in the number of affected children was not, in fact, due to a true, acute rise in the incidence of CNS tumors will require several more years of observation.

The incidence of brain tumors peaks in the first decade of life, then decreases until a second peak in older adulthood. This second peak historically occurred in the seventh decade of life, but recent Surveillance, Epidemiology, and End Results (SEER) data demonstrated a shift in this peak to the eighth decade.[7] The first peak is characterized by a predominance of male patients and by equal incidence rates for whites and blacks, except for the first 2 to 3 years of life, when more whites than nonwhites are affected.[4] The predominance of males is explained mostly by a disproportionate incidence of both medulloblastoma and ependymoma. For other tumor types, the genders are equally affected. During the first 2 years of life, cerebral lesions predominate; cerebellar lesions are more common through the rest of the first decade. The adult pattern of anatomic distribution of tumors is not seen until late adolescence. Within the first decade as well, tumors of embryonal histology are seen much more commonly than in adults. Thus, such tumors as medulloblastoma, supratentorial primitive neuroectodermal tumors (sPNETs), and pineoblastomas occur almost exclusively in children and young adults. High-grade gliomas, including glioblastoma multiforme, are much less common in these age groups than in adults.

Only two factors are consistently noted to place a child at increased risk for a CNS malignancy: various genetic disorders and exposure to ionizing radiation.[8,9]

## ASSOCIATIONS WITH INHERITED SYNDROMES

Fewer than 10% of children with brain tumors will have a syndrome that places them at increased risk for developing a brain tumor. Those syndromes are listed in Table 27-1. Although rare, they place the child at a markedly higher risk for developing other tumors as well. Affected children, therefore, must be monitored closely for evidence of neoplasia both in and outside the CNS.[10] All the syndromes have an autosomal dominant pattern of inheritance, and somatic mutations have been demonstrated in specific genes for each (Table 27-1). Identification of mutations is important to establish cause of the disorders and to provide insights into gene functions.

| Syndrome | Gene(s) | Tumor type(s) | Reference |
|---|---|---|---|
| Cowden | *PTEN* | Dysplastic gangliocytoma of the cerebellum (Lhermitte-Duclos) | 29 |
| Li-Fraumeni | *TP53* | Multiple brain tumor types but most commonly astrocytoma and medulloblastoma | 19, 20 |
| Neurofibromatosis type 1 | *NF-1* | Neurofibroma, optic nerve glioma, astrocytoma | 21, 22 |
| Neurofibromatosis type 2 | *NF-2* | Acoustic and peripheral schwannoma, meningioma, spinal ependymoma | 25, 26 |
| Nevoid basal cell carcinoma | *PTCH* | Medulloblastoma | 15, 16 |
| Tuberous sclerosis | *TSC1, TSC2* | Subependymal giant-cell astrocytoma | 27, 28 |
| Turcot | *APC, hMLH1, hPMS2* | Medulloblastoma, astrocytoma | 17, 18 |
| von Hippel-Lindau | *VHL* | Hemangioblastoma | 30 |

**TABLE 27-1. INHERITED DISORDERS ASSOCIATED WITH BRAIN TUMORS**

Fewer than 5% of children with medulloblastomas have inherited disorders. [11] The most common of these are the nevoid basal cell carcinoma (Gorlin) syndrome and Turcot syndrome.[12,13] The Gorlin syndrome has been linked to germline mutations of the Sonic hedgehog receptor *PTCH*.[14,15 and 16] Affected children are born with multiple skeletal anomalies and macrocephaly. They have a 3% incidence of medulloblastoma, which is diagnosed, on the whole, at a younger age than in children without Gorlin syndrome. These children are predisposed to basal cell carcinomas, a risk that is substantially increased in the fields of radiation used to treat medulloblastoma. Children with Turcot syndrome, an autosomal dominant disorder due to mutation of the *adenomatosis polyposis coli (APC)* gene or to one of several DNA mismatch repair genes (*hPMS2* and *hMLH1,* for example),[17] have a high incidence of colorectal adenomas, gliomas, and medulloblastomas.

Multiple cancer types occur in children with Li-Fraumeni syndrome, caused by germline mutation of the *TP53* gene.[19,20] The protein encoded by this gene is multifunctional, having a role in cell cycle control, in ensuring DNA integrity and repair and, in some circumstances, in inducing apoptotic cell death. Children with inherited mutations of the *TP53* gene most commonly develop low- or high-grade gliomas that may be multifocal, medulloblastomas, PNETs, and choroid plexus tumors. They also have increased incidence of sarcomas, leukemia, and adrenocortical carcinomas.

Children with neurofibromatosis type 1 (NF-1), due to mutation of the *NF-1* gene, are at risk for developing dermal and plexiform neurofibromas and have a markedly increased risk for astrocytomas.[21,22,23 and 24] Most commonly, the astrocytomas occur within the optic pathway as low-grade optic gliomas involving both optic nerves, the chiasm, and the optic radiations. Low-grade gliomas may also occur within the cerebral hemispheres, the brainstem, or the cerebellum. Gliomas and plexiform neurofibromas may undergo malignant transformation. Other cancers have been found to occur in association with NF-1, including myelogenous leukemia, rhabdomyosarcoma, and pheochromocytoma.

Neurofibromatosis type 2, due to mutation of the *NF-2* gene, is associated with meningiomas and schwannomas of the cranial nerves and of the peripheral nervous system.[25 and 26] Bilateral acoustic nerve schwannomas are highly associated with neurofibromatosis type 2. Gliomas and ependymomas also occur with increased frequency and tend to be located in the spinal cord.

Finally, several rare tumor types occur most frequently in association with specific inherited disorders. Subependymal giant-cell astrocytomas in the anteromedial aspect of the brain near the foramina of Monroe most often occur in children with tuberous sclerosis. [27,28] Cerebellar gangliocytoma (Lhermitte-Duclos) occurs in the context of Cowden's syndrome, owing to mutation of the *PTEN* gene that encodes a mixed phosphatase.[29] Hemangioblastomas, typically in the cerebellum, spinal cord, or retinas, occur in association with von Hippel-Lindau syndrome, which arises from mutation of the *VHL* gene that appears to have a role in DNA replication. [30,31 and 32]

## OTHER ASSOCIATIONS WITH CENTRAL NERVOUS SYSTEM TUMORS

### Ionizing Radiation

Exposure to ionizing radiation is a well-documented cause of brain tumors. Children treated with radiotherapy for tinea capitis during the 1940s and 1950s were found to have increased risk for the development of gliomas, meningiomas, and nerve sheath tumors 22 to 34 years later. [33] More recently, brain tumors after cranial irradiation for acute lymphoblastic leukemia have been reported. [34,35,36 and 37]

### Other Cancers

With or without radiotherapy, brain tumors may be seen in association with other cancers or with their treatment. Pituitary tumors occur in patients with various forms of the multiple endocrine adenomatosis syndrome (see Chapter 37). The trilateral retinoblastoma syndrome is pineoblastoma in association with bilateral retinoblastoma.[38] Brain tumors may be seen in a minority of patients with malignant rhabdoid tumors of the kidney. [39]

### Immunosuppression

In various immunosuppression syndromes, such as the Wiskott-Aldrich syndrome, ataxia-telangiectasia, and acquired immunodeficiency, and after solid-organ transplantation, lymphoma of the brain occurs at a frequency higher than that in the normal population. [40,41 and 42]

### Familial Conditions

Data are inconclusive regarding less completely understood familial factors outside of known Li-Fraumeni families. Some studies show no influence of family history on the occurrence of brain tumors, whereas others report an increased risk of brain tumors with a family history of bone cancer, leukemia, and lymphoma. The children or siblings of persons with brain tumors may be at higher risk for developing brain tumors themselves. [43,44,45,46 and 47] Reports of familial clustering of embryonal tumors, gliomas, and CPP also exist.[48,49,50,51,52 and 53]

### Environmental Exposures

The effect of environmental exposures, including diet, on the occurrence of brain tumors has been studied by numerous investigators. [8,54,55,56 and 57] The results of these studies are inconclusive for an association.

Several factors confound the epidemiologic study of pediatric brain tumors: First, until recently, etiologic studies considered pediatric cancer a single entity, and brain tumors were not being examined separately. Second, the etiology of brain tumors most likely is multifactorial, and these factors may influence distinct histologic types of tumors to variable degrees. Finally, pediatric brain tumors are rare, and this rarity affects research methodology.

Nearly all studies of pediatric brain tumors are case-control studies in which individuals with and without brain tumors are compared with respect to past exposures. Inaccuracies and disparities in patient or parent recall may limit observations of disease and their associations. [8] Should a link with environmental exposures truly exist, establishing it may be difficult.

## CENTRAL NERVOUS SYSTEM TUMOR BIOLOGY: TUMOR GENETICS AND CYTOGENETICS

Cancers arise as a result of mutations of genes that regulate cell proliferation and death. Gene mutations may originate within the germline or may occur as somatic mutations exclusively within tumor cells. As noted, only a small fraction of children with brain tumors have germline mutations either acquired from their parents (giving them an inherited predisposition to cancer) or as new mutations. Although the causes of the somatic mutations underlying the vast majority of all brain tumors are

unknown, the genetic abnormalities typically associated with childhood brain tumors are being characterized through a growing body of evidence.

Fluorescence-activated cell sorting and direct chromosomal preparations from biopsy specimens have demonstrated that low-grade gliomas, meningiomas, and pituitary adenomas almost universally possess a unimodal diploid DNA content. [58] In contrast, direct biopsy preparations from more aggressive and malignant tumors, such as anaplastic astrocytoma and glioblastoma multiforme, frequently show bizarre chromosomal aberrations and a dominance of triploid and tetraploid cells in the cell lines derived from these tumors. These findings are in agreement with the genetic analyses of cancers arising in other sites, which generally have demonstrated increasingly abnormal genetic content as cancers evolve into high-grade malignancies. Conversely, fluorescence-activated cell sorting and karyotyping performed on freshly prepared tumor cell populations show near-diploid genetic content, possibly as a result of tumor contamination with normal diploid stromal cells. Alternatively, small diploid or near-diploid anaplastic cells may harbor specific mutations that render them both drug- and radiation-resistant, such that their malignant characteristics are not caused by overt loss or gain of chromatin. [58,59,60,61,62,63,64] and [65]

Chromosomal anomalies have been defined further through molecular genetic analyses, including studies of loss of heterozygosity, comparative genomic hybridization, and fluorescence *in situ* hybridization. The most consistent genetic abnormalities have been found in medulloblastomas and atypical teratoid-rhabdoid tumors (AT/RT).

Deletions of the short arm of chromosome 17 (17p) distal to the *TP53* locus occur in 30% to 50% of medulloblastomas. [66,67] Loss of 17p frequently occurs in association with duplication of 17q, which is characteristic of isochromosome 17q, the most common cytogenetic abnormality of medulloblastomas. [68,69] and [70] Trisomy 7 is the second most common cytogenetic abnormality. [68,70] Deletions or mutations of chromosome 9q involving the Sonic hedgehog receptor *PTCH* have been found in association with approximately 10% to 15% of sporadic medulloblastomas. [71,72,73] and [74] A variety of other chromosomal deletions or additions have been found less consistently. [75,76]

The nonrandom occurrence of monosomy 22 is common in several neural tumors, including medulloblastomas, ependymomas, meningiomas, acoustic neuromas, and AT/RT. [63,77] Mutations or deletions of chromosome 22q11.2 involving the *hSNF5/INI1* gene are present in nearly all AT/RT and in rhabdoid tumors outside the CNS. [76,78] The function of the protein encoded by this gene is incompletely understood. It forms part of the SWI/SNF complex that apparently alters chromatin structure to allow gene transcription. [79] In addition to monosomy 22, deletions of 6q in ependymomas have been described by several investigators. [80,81]

A variety of genetic events have been associated with the progression of low-grade gliomas to high-grade glioblastomas in adults: nonrandom loss of chromosome 10 or of portions of 9p, 17p, and others; gene amplifications of, for example, *EGF* or *MDM2*; and mutations of such specific genes as *PTEN* and *TP53*. [58,82,83] and [84] These observations suggest that, as in colon carcinoma, malignant progression of CNS tumors may be a multistep process involving the accumulation of genetic abnormalities that promote activation of multiple dominant oncogenes and inactivation of recessive tumor suppressor genes. [67,85,86,87,88,89,90] and [91]

## PATHOLOGIC CLASSIFICATION OF CENTRAL NERVOUS SYSTEM TUMORS

### Background

Classification of CNS tumors has been a challenge for more than 100 years. Almost from the beginning, several different histologic classifications have coexisted, reflecting a lack of consensus among neuropathologists. Historical details of this problem have been reviewed in detail by Zulch. [92] All classification schemes are basically arbitrary, hypothetical statements that may or may not reflect the natural order of the things being classified. Ideally, however, they change with progress in knowledge, are clinically relevant, and provide a structure within which a subject may be arranged. These goals may not necessarily be achieved by the same scheme.

Classification of CNS tumors became important clinically after successful establishment of neurosurgery as a specialty and received major impetus from Harvey Cushing during the 1920s and 1930s. The classification system proposed by Cushing's student, Percival Bailey, in 1925 has served as the prototype system since then. That scheme was based on the cell-of-origin notion introduced by German pathologists. They suggested that tumors developed from cells arrested at various stages of development; for each putative developmental stage of a cell, a corresponding tumor was identified. Figure 27-2 outlines Bailey and Cushing's basic schema, denoting the different cell types, the developmental stages through which they were said to pass before reaching maturity, and the corresponding tumor types presumed to arise from them (noted in parentheses in the figure). This classification was embraced immediately because it reflected some aspects of clinical behavior and prognosis, corresponding to Cushing's experience, and because of the esteem that Cushing enjoyed.

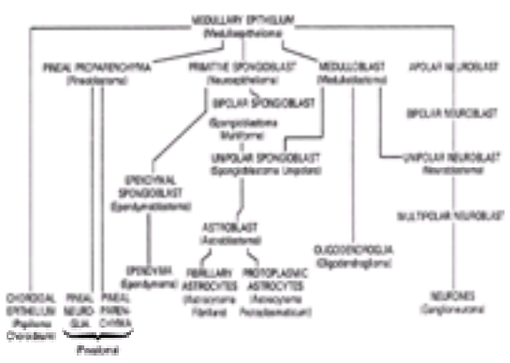



**FIGURE 27-2.** Bailey and Cushing schema of normal developing cells and neuroepithelial tumors derived from them.

### Methods of Classification

#### *Morphologic and Histogenetic Classification*

In addition to their cell-of-origin concept, Bailey and Cushing recognized that tumors were composed of heterogeneous cells. They decided to classify tumors on the basis of the morphology and presumed histogenesis of the *predominant* cell type. Hence, if the majority of cells resembled astrocytes, the tumor was called an *astrocytoma*, even though a small number of other cells (e.g., oligodendrocytes) also were present. Most classifications in current use are based on this concept.

Approximately 60 years ago, Kernohan et al. [93] introduced a grading system based on the concept that glial cells of all types become progressively more anaplastic over time. Criteria were advanced for grading glial tumors on a scale from 1 (most benign) to 4 (most malignant). The scheme was to be applied to astrocytomas, oligodendrogliomas, and ependymomas (Table 27-2). Based on histologic features, it has been adopted readily and is used commonly for astrocytomas for which clinical correlations exist. For the other two tumor types, the scheme has been an awkward fit and, as a result, never has been used consistently for them. Revision of the Kernohan grading system for astrocytomas has been proposed by Daumas-Duport et al. [94]

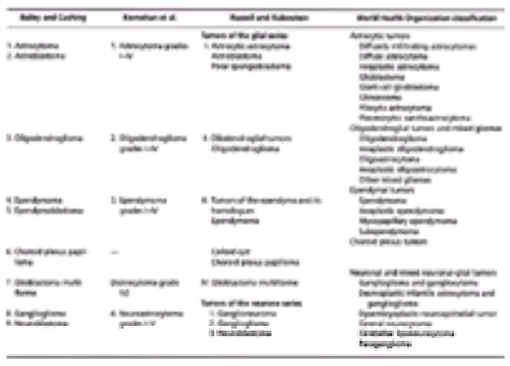


**TABLE 27-2. COMPARISON OF CLASSIFICATION SYSTEMS FOR CENTRAL NERVOUS SYSTEM TUMORS**

An international panel of neuropathologists working under the aegis of the WHO has expanded the concept of grading to all CNS tumors, using the 1-to-4 scale to indicate biologic malignancy.[95] Table 27-2 provides a comparison of four morphologic classification systems: (a) Bailey-Cushing, (b) Russell-Rubinstein, (c) Kernohan et al., and (d) the most recent revision proposed by the WHO.[10] The 1989 Russell-Rubinstein classification is a reorganization of that proposed by Bailey and Cushing. A revision of the Russell-Rubinstein text by Bigner et al.[96] has been advanced too recently to allow its usefulness. Rorke et al.[97,98] proposed and recently modified a classification scheme that recognizes not only morphologic entities and degree of anaplasia but tumor location as well. The proposed histologic reclassification, shown in Table 27-3, is based on advances in identifying cell types by use of immunoperoxidase methods that allow more precision in the categorization of tumors. Tumor location (data not shown) is designated by a Roman numeral and letter: For example, thalamus or basal ganglia (or both) are designated *Ib*, and cerebellum is *Ii*. By considering location, this scheme acknowledges the importance of site of origin as a factor in determining clinical outcome. The most obvious example is the pilocytic astrocytoma; a child with a lesion in the cerebellum generally has a better prognosis than one whose lesion is in the diencephalon.

**TABLE 27-3. PROPOSED MODIFICATION OF REVISION OF THE WORLD HEALTH ORGANIZATION CLASSIFICATION OF BRAIN TUMORS IN CHILDREN (NEUROEPITHELIAL TUMORS ONLY)**

### *Pitfalls of Morphologic and Histogenetic Classification*

It is important to recognize that the histogenetic concepts underlying the morphologic classification schemes are not fully tenable in light of current knowledge.[99,100] It has long been established that cancer is a genetic disease in which the genotypic instability of neoplastic cells may change the histologic features consequent to both time and treatment. At the same time, a large proportion of tumors exhibit relatively characteristic features, allowing easy diagnosis to the trained eye. Until a more accurate basis for classification of CNS tumors emerges, the morphologic approach must be used.

It has been established that cell populations displaying similar, even identical, patterns of differentiation may not have a common embryogenesis as theorized by Bailey and Cushing.[101] Therefore, it is not possible to determine accurately either ancestry or progeny of a tumor cell or cells. Furthermore, the assumption that one or a few histologic features adequately predict clinical behavior of tumors without consideration of other factors, such as site of origin, is untenable. Gilles et al.[102] compared three separate cerebellar tumor types—medulloblastoma, astrocytoma, and ependymoma—with different prognoses and found a broad overlap of multiple, discretely identifiable histologic features.

### *Phenotypic Classification*

An alternative to classification based on histogenetic concepts is the phenotypic approach. Here, immunohistochemistry and techniques of molecular biology are used to assist in identification of cell types comprising the tumor.[103,104] and [105] Use of these techniques to complement standard light and electron microscopy provides more objective identification of cell types, thus allowing more precise classification of CNS tumors. The use of monoclonal antibodies to identify specific antigens, such as cytoskeletal and membrane proteins, hormonal polypeptides, and neurotransmitter substances, has been especially useful in classifying, on routine light microscopy, tumors with unusual morphologic features that previously were relegated to the “unknown” category. In particular, it has allowed greater understanding of the biology of embryonal tumors, as the differentiating potential of the primitive cells can be identified.[106,107]

Use of this phenotypic approach led to identification and separation of the AT/RT from PNET or medulloblastoma, a distinction that appears to have clinical importance.[108] Pathologic features of this tumor are complex and have presented diagnostic challenges to pathologists who most frequently had diagnosed AT/RT as medulloblastoma or PNET or, rarely, as choroid plexus carcinoma (CPC). All tumors in this group, by definition, contain rhabdoid cells. They are round to oval and medium-sized and typically have an eccentric nucleus containing a prominent nucleolus. Cytoplasm is finely granular or homogeneous and sometimes contains a dense, pink, poorly defined “mass” suggesting an inclusion body. Cell borders tend to be distinct. Approximately two-thirds of these tumors contain fields indistinguishable from PNET, and one-third contain malignant mesenchymal elements, whereas one-fourth exhibit fields of epithelial cells that may be glandular or, rarely, squamous. Field necrosis is common, and mitotic figures generally are abundant.

Immunohistochemical features of AT/RT are complex and vary depending on the cellular composition of the tumors. In general, rhabdoid cells express vimentin and epithelial membrane antigen and (less frequently) smooth muscle actin. These cells also may express neurofilament proteins (NFP), glial fibrillary acidic protein (GFAP), and keratin. The cells comprising the PNET component may express NFP, GFAP, desmin, or no antigen, whereas the epithelial portions express keratin and epithelial membrane antigen or vimentin (or both). The mesenchymal cells express vimentin, sometimes smooth muscle actin, and rarely desmin.

Use of immunoperoxidase techniques with various antibodies has forced reconsideration also of the nosology of certain tumors long regarded as astrocytomas, specifically subependymal giant-cell astrocytoma, pleomorphic xanthoastrocytoma, and superficial cerebral astrocytoma. In 1980, Bender and Yunis[109] called attention to the presence in subependymal giant-cell astrocytomas of cells that expressed neurofilament protein, an observation confirmed by Nakamura and Becker[110] and by Bonnin et al.[111] In fact, the majority of cells composing these tumors expresses vimentin, whereas only a few express GFAP, and some tumor cells actually coexpress both NFP and GFAP.[110] The expression of either of the neuronal markers would rarely, if ever, be seen in pure astrocytomas.

Powell et al.[112] documented expression of neurofilament protein in a variable number of cells in pleomorphic xanthoastrocytoma and noted that some of these tumors also contain foci that resemble frank ganglioglioma.[112,113]

It has become apparent that the tumor described by Taratutoet al.[114] in 1984 as a dural astrocytoma and the desmoplastic infantile ganglioglioma (DIG) defined in

1987 by VandenBerg et al.[115] really belong in the same category, as both express GFAP and NFP and exhibit a prominent desmoplastic component.[116] These observations form the basis of Rorke's removal of these three tumors from the astrocytoma category and their placement within the glial-neuronal neoplasms ( Table 27-3).

For optimal study, tumor tissues removed at biopsy and post mortem require special handling. If a tumor is in an accessible location, the surgeon should be encouraged to remove and submit as much tissue as is safely possible; post mortem, generally no limitations apply. At any rate, a fresh sample should be sent for cytogenetic studies, a piece should be frozen, some should be fixed in formalin, tiny fragments should be fixed in glutaraldehyde in the event that ultrastructural study is required, and the bulk of the specimen should be placed in Bouin's fixative. Although this is ideal for preserving cell morphology, fixation in Bouin's creates three disadvantages, including the following: (a) cytogenetic studies cannot be performed, (b) synaptophysin antigen cannot be retrieved, and (c) the tissues cannot be tested for B-cell markers in the event that a lymphoma is suspected.

High-quality technical preparations are of utmost importance in establishing a diagnosis. Use of the microwave enhancement technique for certain antigens is recommended.[117,118] Even under the most optimal circumstances, however, classification based on phenotypes also may be problematic.

Table 27-4 contains a listing of the widely available markers used most commonly and their utility in the differential diagnosis of tumors arising in the CNS. These tumors include primary neuroepithelial tumors, those arising from meningeal coverings, and germ cell tumors.

| Marker | Tumor types containing positive cells |
|---|---|
| Glial fibrillary acidic protein | Astrocytoma, ependymoma, mixed glioma, gliosarcoma, ganglioglioma, gliofibroma, multiforme, glioflorma; positive cells occasionally may be found in oligodendroglioma, capillary hemangioblastoma, choroid plexus papilloma, PNET, AT/RT |
| Neurofilament | Ganglioglioma, gangliocytoma, PNET, neurocytoma, subependymal giant cell tumor, AT/RT |
| Vimentin | Mesenchymal tumors, meningioma, sarcoma, melanoma, lymphoma, ependymoma, astrocytoma, glioflorma, chordoma, schwannoma, hemangioblastoma, carcinoma, PNET, AT/RT |
| S100 and neuron-specific enolase | Positive in a variety of normal and neoplastic cells of neural and nonneural origin; of questionable utility for diagnostic purposes |
| Desmin | Tumors containing muscle (rhabdomyosarcoma, teratoma, etc.), PNET |
| Cytokeratin | Chordoma, choroid plexus tumors, meningioma, certain anaplastic gliomas, nongerminomatous germ cell tumors, PNET, AT/RT |
| Epithelial membrane antigen | Meningioma, ependymoma, epithelial areas of teratomas, rhabdoid tumor (AT/RT) |
| Synaptophysin | PNET, ganglioglioma, gangliocytoma, central neurocytoma, neuroblastoma tumors |
| Smooth muscle actin | Tumors containing muscle, AT/RT |
| Retinal S-antigen | Pineal parenchymal tumors, PNETs, retinoblastoma |
| Alpha fetoprotein | Embryonal carcinoma, endodermal sinus (yolk sac) tumor |
| Human chorionic gonadotrophin | Germinoma, choriocarcinoma |
| Placental alkaline phosphatase | Germ cell tumors |

AT/RT, atypical teratoid rhabdoid tumor; PNET, primitive neuroectodermal tumor.

**TABLE 27-4. COMMON MARKERS FOR DIAGNOSIS OF CENTRAL NERVOUS SYSTEM TUMORS**

### Unsolved Issues in Classification of Embryonal Tumors of Neuroepithelial Origin

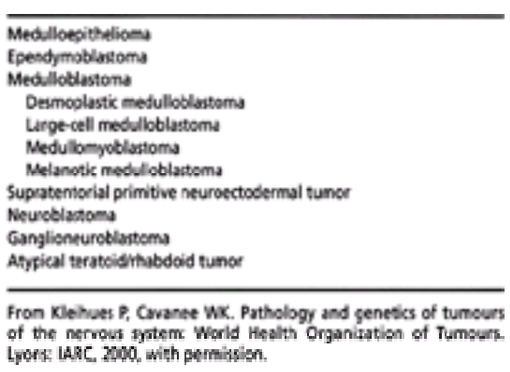
From the time that the prototypic CNS embryonal tumor—medulloblastoma—was described by Bailey and Cushing, controversy has swirled around its nature, origin, and name.[119] A historical summary of the issues may be found in a number of papers that interested readers should consult. [120,121,122] and [123]

The essential issue at the heart of the controversy is whether the medulloblastoma is a tumor unique to the cerebellum. If tumors of their histology and biologic behavior occurred only in the cerebellum, no problem would exist. In reality, however, histologically and biologically identical tumors may arise in the cerebrum, the pineal and suprasellar regions, the spinal cord, and the brainstem. Such tumors were well known to Bailey and Cushing who, however, remained ambivalent in regard to what name should be given to the extracerebellar tumors resembling medulloblastomas.[124] In consequence, a large number of diagnostic terms have been applied to medulloblastoma-like tumors that arise outside the cerebellum.

A proposal to resolve the problem in 1983 seemed, instead, to stimulate further controversy.[121] Specifically, it was proposed that tumors composed primarily of apparently undifferentiated neuroepithelial cells be considered a unique diagnostic group regardless of site of origin in the CNS, recognizing that the majority of such tumors do, in fact, arise in the cerebellum. Introduced by Hart and Earle, the diagnostic term *primitive neuroectodermal tumor* (i.e., PNET) was suggested as appropriate for this group, with the addition of modifiers specifying differentiation along one or more lines (e.g., neuronal, glial) if such were identified by use of phenotypic markers (i.e., antibodies for NFP, GFAP, etc.).[125] This proposal was criticized as a “simplistic” approach that would foster intellectual languor among pathologists who would use the diagnosis of PNET as a catchall term for all difficult-to-diagnose tumors primarily composed of poorly differentiated neuroepithelial cells.[126] Also, many were reluctant to abandon the diagnostic term *medulloblastoma* because of its familiarity and association with an enormous body of medical literature.

Conversely, the science on which the term *medulloblastoma* is based is faulty. The practice of using a special diagnostic term for a tumor in a specific location flies in the face of the fact that diagnosis of all other types of CNS tumors generally is based on histologic features and not on the site of origin. For example, a pilocytic astrocytoma in the cerebellum, diencephalon, or spinal cord is called a *pilocytic astrocytoma* regardless of the different locations. In contrast, if a tumor with the histologic features of medulloblastoma arises in the cerebrum or pineal, the current practice is to call it a *supratentorial PNET* (sPNET) or *pineoblastoma*, respectively. However, if the location is not known, a pathologist is forced to make a diagnosis of medulloblastoma, as no distinguishing histologic features separate embryonal tumors of this type, which may occur anywhere in the CNS (including the pineal), from those that arise in the cerebellum.

In 1993, a group of international neuropathologists decided to include the umbrella term *primitive neuroectodermal tumor* as a category under embryonal tumors of the CNS and then designated medulloblastoma as one type of PNET.[95] However, two subsequent revisions of the classification of CNS tumors have undone that categorization, such that the most recent WHO classification of embryonal tumors limits medulloblastomas to the cerebellum and contains a category of sPNET to deal with such tumors in the cerebrum (Table 27-5).[10] Orphaned in terms of diagnostic classification are those rare PNETs that originate in the suprasellar region, brainstem, and spinal cord. Pineal PNETs are still called *pineoblastomas*, and the designation *ependymoblastoma* also has been retained by the WHO committee. The current classification distinguishes the rare variant forms of PNETs, which contain muscle (or at least express muscle antigens) or melanin or both; these tumors are rarely encountered in pediatric neuropathology.



Medulloepithelioma
Ependymoblastoma
Medulloblastoma
  Desmoplastic medulloblastoma
  Large-cell medulloblastoma
  Medullomyoblastoma
  Melanotic medulloblastoma
Supratentorial primitive neuroectodermal tumor
Neuroblastoma
Ganglioneuroblastoma
Atypical teratoid/rhabdoid tumor

From Kleihues P, Cavanee WK. Pathology and genetics of tumours of the nervous system: World Health Organization of Tumours. Lyons: IARC, 2000, with permission.

**TABLE 27-5. 2000 CLASSIFICATION OF EMBRYONAL TUMORS OF THE CENTRAL NERVOUS SYSTEM BY A WORLD HEALTH ORGANIZATION COMMITTEE**

Highly likely is that the classification of pediatric CNS tumors will continue to evolve. Expanding studies of tumor cytogenetics raise the hope that CNS neoplasms will be able to be classified on the basis of unique cytogenetic features, leaving aside the difficult issues of morphology. Whereas considerable progress has been made along this path (see Kleihues and Cavanee[10]), a great deal of work remains to be done.

## CLINICAL PRESENTATION: NEUROLOGY OF CENTRAL NERVOUS SYSTEM TUMORS

No single clinical finding is pathognomonic for the diagnosis of a childhood brain tumor. At the onset of illness, the nature of neurologic and systemic dysfunction is varied. The presentation relates mostly to the site of tumor origin, affected children's ages, and their developmental level, but sometimes depends also on the tumor type. Clinical prodromes may include features of increased intracranial pressure (ICP), symptoms and signs of a localizing nature, or symptoms and signs without a localizing quality.

### Increased Intracranial Pressure

Brain tumors cause increased ICP directly by infiltrating or compressing normal CNS structures or indirectly by causing obstruction of cerebrospinal fluid (CSF) pathways and resulting in noncommunicating hydrocephalus. Initial features of elevated ICP often are insidious, nonspecific, and nonlocalizing. Among school-aged children, declining academic performance, fatigue, personality changes, and vague intermittent headaches are common. Over time, morning headaches, vomiting, and lethargy ensue. Papilledema may develop if the pressure is long standing. Rapid progression of symptoms secondary to increased ICP is infrequent but, when such occurs, a quickly growing midline or posterior fossa tumor requiring immediate intervention should be suspected.

Brain tumor headaches frequently have ominous features distinct from tension headaches or migraines.[127,128] When children with a tumor are recumbent, increased ICP often will worsen, and the resulting pain may wake them at night or accompany them on waking in the morning. On arising, vomiting may occur along with some relief of pain. Once such patients are upright, the headache diminishes over the course of the morning. Over time, headaches gradually increase in severity and frequency and clearly differ from any previous pain. The pain, which usually is frontal or occipital rather than temporal, may be exacerbated further with Valsalva maneuvers. The clinical suspicion for tumor should be greatest in those children with recent and continuing complaints of headache, and such should prompt a careful history and evaluation for related symptoms and signs. In fact, by 6 months from headache onset, nearly 100% of children have associated neurologic signs, such as papilledema, strabismus, ataxia, or weakness.[127]

For infants and young children whose skulls may more easily accommodate the growth of a mass lesion, the presentation of elevated ICP may differ. Irritability, anorexia, failure to thrive, and even developmental regression can be frequent early signs. Chronically increased pressure may lead to macrocephaly and splitting of the cranial sutures. An infant ultimately may develop a tense or bulging anterior fontanelle. A baby may develop a shrill, neurogenic cry. Funduscopic evaluation of these patients may reveal only optic pallor and no evidence of papilledema. The setting-sun sign, a seemingly forced downward deviation of the eyes and part of Parinaud's syndrome, may be seen.

Parinaud's syndrome is a collection of ophthalmologic findings stemming from increased ICP at the dorsal midbrain. Beyond the impaired upward gaze seen in infants, older children also display large pupils with impaired reflex constriction to light but not with accommodation. Convergence of gaze may evoke repetitive, bilateral, adducting nystagmus with retraction of the globes in the orbit. A nerve IV palsy, with the affected eye deviated upward and laterally, also may occur. Affected children often compensate for the trochlear nerve palsy by tilting their heads toward the shoulder of the unaffected eye.

A head tilt may occur also with increased ICP because of a stiff neck and cervical root irritation from incipient cerebellar herniation of a posterior fossa mass. Other signs of increased ICP include listlessness and horizontal diplopia from pressure on the long, free intracranial course of the abducens nerve.

### Localizing Symptoms and Signs

Children with supratentorial tumors (i.e., of the cerebrum, basal ganglia, thalamus, hypothalamus, and optic chiasm) may demonstrate various localizing symptoms and signs, depending on the tumor size and its specific origin. Many of these signs precede those of increased ICP. The most common of these signs are hemiparesis, hemisensory loss, hyperreflexia, seizures, and visual complaints.

Vision loss may localize to any location in the optic pathway. Complaints occasionally start insidiously with such events as a failed school eye examination or a need for eyeglasses. Tumors confined to the optic nerve produce monocular vision loss. Chiasmatic tumors present often with a complex visual field loss and decrement in acuity, whereas lesions located more posteriorly, in the optic tract, lateral geniculate nucleus, optic radiations, or occipital cortex, demonstrate some aspect of hemianopsia.[129] A paradoxical increase in pupillary size to light when moving the source from one eye to the other indicates a relative afferent pupillary defect (the Marcus-Gunn pupil), a potential sign of tumor at the optic nerve or chiasm. Among infants, chiasmatic tumors may result in unilateral or bilateral pendular nystagmus, with head nodding and head tilt, a triad known as *spasmus nutans*.[130]

In contrast to the experience in adults, a seizure is seldom the sine qua non of a supratentorial mass in children. Nevertheless, all simple and complex partial (i.e., focal) seizures and most unexplained generalized (grand mal) seizures mandate computed tomography (CT) or MRI of the brain. After a first seizure and subsequent CT, fewer than 1% of patients are given diagnoses of a tumor.[131] Certain features of a seizure or seizures are associated with an increased risk of a tumor. They include a change in the character of the preexisting seizures, status epilepticus as the first seizure, prolonged postictal paralysis, resistance to medical control, and the presence of focal symptoms or deficits.[132,133 and 134] An initially normal CT scan in patients with these seizure characteristics or with persistent epilepsy does not rule out the possibility of a tumor, and repeat imaging with MRI may be indicated.

Other localizing signs for a supratentorial tumor may be subtler. Children with frontal lobe tumors may have a long history of behavioral problems. Tumors that involve the hypothalamus may cause minimal or no motor or visual difficulties. In infants, these tumors may cause failure to thrive and emaciation with a paradoxical euphoric mood and increased appetite, the so-called diencephalic syndrome.[135] By age 18 to 24 months, most children have a right- or left-hand dominance; earlier dominance or subsequent change in handedness implies lateralized disease.

For infratentorial tumors—those arising from the cerebellum and brainstem—localizing features include ataxia, long-tract signs, or cranial neuropathies. Initial cerebellar dysfunction may be insidious, with clumsiness, worsening handwriting, difficulty with hopping or running, or slow or halting speech. Tumors arising in the cerebellar hemispheres more commonly cause lateralizing signs, such as appendicular dysmetria and nystagmus, whereas midline cerebellar masses lead to truncal unsteadiness or increased ICP.

Cranial neuropathies often suggest brainstem pathology. Diplopia, with images seen side by side, is common from invasion of the abducens nerve within the pons. Inability to abduct one or both eyes (abducens palsy), however, can be a false localizing sign, as it may result also from increased ICP trapping of the abducens nerve against the edge of the tentorium. Inability to deviate both eyes conjugately (gaze palsy) or the inability to adduct one eye properly on attempted lateral gaze implies an intrinsic brainstem disorder. These latter findings alone or, more likely, in combination with deficits of the trigeminal, facial, or auditory nerve strongly suggest tumor involving the brainstem. Indeed, masses involving the cerebellopontine angle may result in facial weakness, absent corneal reflex, and hearing loss. Weakness of an entire half of the face (peripheral seventh nerve palsy) suggests a posterior fossa tumor; weakness of the lower face on one side, with spared eyelid closure and forehead movement (central seventh nerve palsy), suggests involvement anywhere superior to the pons. Drooling and swallowing difficulties may arise from involvement of the medulla. A partial Horner's syndrome (ipsilateral ptosis, miosis, and anhidrosis) may be present also in some patients with hypothalamic, brainstem, or upper cervical cord disease, as a result of compromise of the descending sympathetic tracts.

### Nonlocalizing Symptoms and Signs

Some symptoms are characteristic of a brain tumor but not specifically localizing. Affected children may display changes in affect, energy level, motivation, or behavior. They may exhibit weight gain or loss with anorexia. Sexual precocity or delayed puberty, growth failure, somnolence, or symptoms of an autonomic nature may suggest hypothalamic or pituitary dysfunction or may be nonspecific. Vomiting can occur with irritation of the area postrema in the floor of the fourth ventricle from a generalized increase in ICP or from direct irritation by a mass.

As many as 15% of primary CNS tumors, particularly medulloblastoma, germ cell tumors, ependymoma, and high-grade gliomas, have disseminated to other CNS sites by the time of diagnosis.[136] Although such dissemination usually is asymptomatic, neurologic dysfunction from such lesions sometimes overshadows the symptoms of the primary tumor, confusing the localization of tumor origin. For example, spinal cord and cauda equina involvement may cause back or radicular pain, bowel or bladder dysfunction, or long-tract symptoms. Thus, examination at the time of diagnosis should include a search for local tenderness of the spine, focal

extremity weakness, or sensory loss.

### Syndromes Specific to Tumor Types

Although a pathologic brain tumor diagnosis requires tissue biopsy, certain patterns of symptoms and signs are particularly suggestive of specific tumor histologies. In the suprasellar region, pilocytic astrocytomas of the optic pathway and hypothalamus may manifest visual field loss, nystagmus, and diencephalic syndrome but lack any frank endocrinologic abnormality in two-thirds of cases.[137] Craniopharyngiomas also occur in the suprasellar and sellar regions, but these neoplasms present more often with both visual deficits and endocrinopathies.[138] Short stature and diabetes insipidus are frequent disturbances.[139] The endocrinopathies may be obscured, however, if increased ICP and hydrocephalus from obstruction of the third ventricle and foramen of Monro are present.

Germ cell tumors may occur in the anterior hypothalamus as well. These tumors frequently cause long-standing endocrinologic abnormalities, particularly growth failure and diabetes insipidus. Endocrine disturbances may precede the diagnosis by several years. Emotional and behavioral disturbances also can occur.

Germ cell tumors may arise also in the pineal region, as can the pineal parenchymal tumors, pineoblastoma and pineocytoma. All these tumors are apt to be associated with Parinaud's syndrome. Focal motor deficits appear more commonly with infiltrating glial tumors at the pineal region.[140]

In the posterior fossa, brainstem glioma, medulloblastoma, ependymoma, and pilocytic astrocytoma form the oncologic differential diagnosis. Medulloblastoma and ependymoma often compress the fourth ventricle, leading to findings of increased ICP. Vomiting may be extreme with ependymoma because of invasion of the area postrema, an emetic chemoreceptor on the dorsal medulla that protrudes into the fourth ventricle. The classic brainstem glioma, a diffusely infiltrative pontine glioma, presents with a prodrome of less than 6 months consisting of a triad of long-tract signs, ataxia, and cranial neuropathies, particularly an abducens palsy.[141,142] The atypical, focal brainstem glioma presents with a longer prodrome, often without abducens palsy. Cerebellar pilocytic astrocytomas frequently present first with vague symptoms and then with ataxia of long duration, usually a period of 18 months.[143] In the rare cerebellar hemangioblastoma, an elevated hemoglobin level may be noted, secondary to extramedullary hematopoiesis.[144]

Whereas a single seizure seldom is the presenting symptom for histologically malignant cerebral tumors, long-standing epilepsy may be associated with low-grade neoplasms.[132,133] and [134,145,146] In children with long-standing epilepsy found to harbor a tumor, the most common diagnoses are ganglioglioma, dysembryoplastic neuroepithelial tumor (DNET), oligodendroglioma, and LGAs.[147,148] Tumors are found in 12% to 33% of children who undergo surgery for intractable seizures.[149]

Among infants with brain tumors, seizures may occur in conjunction with macrocephaly as the harbinger of DIG, a massive, cystic, and malignant-appearing tumor with a favorable prognosis.[150] CPP presents during infancy with hydrocephalus in nearly all cases. In congenital brain tumors, the most common diagnoses are malignant astrocytoma, teratoma, embryonal tumors, and CPP.[151] For those tumors diagnosed within 2 months of birth, the mass occupies more than one-third of the intracranial volume in 75% of patients.

## NEUROIMAGING IN PEDIATRIC CENTRAL NERVOUS SYSTEM TUMORS: CURRENT STATUS AND FUTURE DIRECTIONS

### Magnetic Resonance Imaging

Preoperative assessment of tumor type and extent, by imaging, is based on the combination of anatomic location, tissue characterization, and enhancement pattern, taken in conjunction with the clinical history. Since its introduction into clinical practice, MRI has superseded CT as the diagnostic tool of choice for pediatric brain and spinal cord tumors. Advantages of MRI include the ease of imaging in three orthogonal planes without the need to move the patient, imaging without the use of x-irradiation, and improved soft tissue contrast. Nevertheless, the clinical presentation of children with brain tumors most often leads to initial evaluation by unenhanced CT. Where MRI is readily available in a timely fashion, CT with iodinated contrast is not recommended because of its inferiority in delineating tumor extent as compared with gadolinium-enhanced MRI.

Routine MRI sequences include T1-weighted imaging (T1WI) before and after gadolinium and T2- and proton density–weighted imaging. Postgadolinium imaging usually is performed with magnetization transfer suppression, which amplifies contrast enhancement by suppressing the signal intensity of normal background brain tissue. As a result, the detection of contrast enhancement is increased by a factor of two to three.[152] This can be useful in demonstrating enhancement within a tumor, extension of the tumor along white-matter pathways, and the subarachnoid spread of tumor. Notable is that contrast enhancement is a reflection not of vascularity but of breakdown of the blood–brain barrier (BBB) and, given this factor, neither CT nor MRI defines the true extent of tumor spread.

MRI offers other advantages over CT scanning. Through the use of the fluid-attenuated inversion recovery sequence, the penumbra of edema surrounding a tumor, which may contain metastatic foci, can be delineated by MRI. This sequence may be useful to the radiation oncologist for targeting focal therapy, although it tends to overestimate the extent of tumor. The introduction of fast-echo planar imaging has enabled the development of diffusion and perfusion techniques, discussed later in this section. Finally, with the advent of frameless stereotaxy, MRI has superseded CT for preoperative planning by virtue of the capability to acquire three-dimensional volumetric data that can be reformatted, in any plane, in the operating room, for tumor localization in relation to markers placed on the skin.

Because it has been replaced by magnetic resonance angiography, conventional angiography rarely is performed in pediatric CNS tumors. Digital subtraction angiography still may be indicated, however, in those cases displaying a mass with blood and a differential diagnosis of vascular malformation versus hemorrhagic tumor. Also, if a highly vascular tumor is suspected, diagnostic angiography may be performed as part of a neuro-interventional procedure before resection to minimize blood loss.

### Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) provides measurement of metabolites within tissue under investigation. For example, proton MRS (HMRS) determines in both a qualitative and a quantitative fashion the chemical environment of the hydrogen nuclei within the tissues targeted. Frequency-domain spectra, which reflect the distribution of resonance frequency of the particular nuclei in the sample, form the data for analysis. Spectra are represented by a series of peaks with positions expressed in parts per million (ppm); the result can be considered a histogram of nuclei with different precession frequencies.

Spectra can be acquired using a single- or multivoxel technique, with short (10- to 30-millisecond) or long (135- to 280-millisecond) echo times. In using a short echo time, more peaks are captured, but the spectrum is superimposed by a complicated baseline, and its analysis is more difficult. With longer echo times, fewer peaks are captured, but the measurement precision is improved. In pediatric brain tumors, the three most important metabolic peaks are, reading from right to left ( Fig. 27-3), *N*-acetyl aspartate (NAA), 2.02 ppm; creatine-phosphocreatine (Cr/PCr), 3.02 ppm; and choline (Cho), 3.22 ppm.

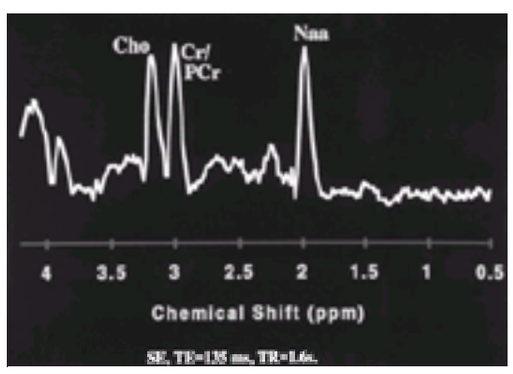



**FIGURE 27-3.** Normal long-echo single-voxel spectrum from the cerebellum of an age-matched control for a patient with medulloblastoma (see Fig. 27-8). Reading from right to left, note normal peaks of *N*-acetyl aspartate (Naa), creatine-phosphocreatine (Cr/PCr), and choline (Cho).

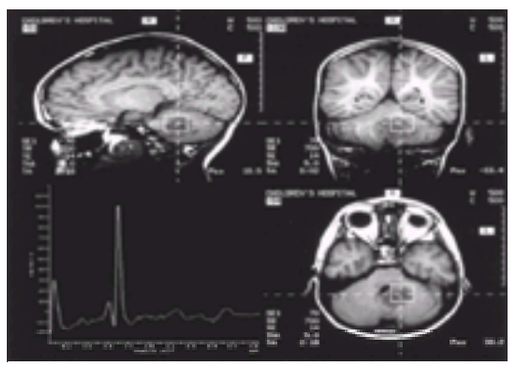


**FIGURE 27-8.** Composite image demonstrating the set-up of a single voxel of interest in all three planes for long echo time (TE) magnetic resonance spectroscopy in a posterior fossa tumor. Note almost complete absence of *N*-acetyl aspartate at 2.02 ppm, loss of creatine-phosphocreatine peak at 3.02 ppm, and gross elevation of choline at 3.22 ppm. Histopathology confirmed a medulloblastoma (primitive neuroectodermal tumor).

NAA is a marker of neuronal and axonal integrity. Cr is a marker for energy metabolism. Cho is a marker for cell membrane turnover and, as such, is elevated in tumors, demyelination, and inflammation; it is decreased in liver disease. Notably, the relative ratios of different metabolites vary, depending on the location of the voxel in the brain and on age during the first 5 years of life, when myelination of the immature brain increases. It is imperative, therefore, to have normal age-matched control data from the same brain region in interpreting the spectra from young children. As a general rule, the NAA increases over time, especially during the first 18 months of life, whereas the Cho slightly decreases over the same period. Creatine-phosphocreatine tends to remain rather stable over time; for this reason, it has been used historically as an internal control when metabolic data are expressed as ratios.

The finding of a lipid-lactate peak usually indicates the presence of ischemia or necrosis. Phosphorus 31 MRS has been used in head and neck tumors, especially in the diagnosis and management of lymphoma. It is more time-consuming and a less generally available technique at present. Fluorine MRS has been used to research tumor drug pharmacokinetics, such as 5-fluorouracil. The uptake of drug in the tissue of interest and its subsequent metabolism can be followed.

Single-voxel MRS can be used to interrogate new tumors having a volume greater than 1 cc. However, a multivoxel technique, such as two-dimensional chemical shift imaging, in which several subcentimeter voxels can be examined simultaneously, can be very helpful in distinguishing recurrent tumor from radiation necrosis. From a compilation of two-dimensional chemical shift imaging spectra, one can obtain a three-dimensional MRS data set. For the future, whole-brain single-metabolite techniques are being developed,[153] which, in addition to double-quantum spectroscopy, will further and more accurately separate out the metabolic peaks.

### Diffusion-Weighted Imaging

Diffusion-weighted imaging (DWI) describes brownian, or random, motion of water molecules. However, in the intracellular and extracellular spaces of the brain, macromolecular proteins, intracellular organelles, cell walls, and myelin sheaths restrict or slow diffusion in certain directions. This restriction results in a new directionality of diffusion within given regions of the brain. This directionality, or anisotropy, is most noticeable in the white-matter tracts in which diffusion tends to be much faster in the direction parallel to myelinated axons. Images can be made for individual directions, usually corresponding to the three orthogonal imaging planes: axial, coronal, and sagittal. Alternatively, the individual directional images can be averaged to produce a relatively direction-independent, or isotropic, diffusion image called a *trace image*. From this, a mean apparent diffusion coefficient (ADC) map can be calculated.

### Magnetic Resonance Perfusion Imaging

In addition to DWI, the development of fast-echo planar imaging has made possible an assessment of the vascularity of tumors using a gadolinium first-pass bolus technique. This relies on changes in the T2 signal of gadolinium-laden blood as it passes through the region of interest. [154] Resulting data, reflected in maps of relative cerebral blood volume, provide some semiquantitative analysis of the blood flow to a particular region. Early work suggested a positive correlation between relative cerebral blood volume and tumor grade.[155] Perfusion imaging may be helpful in targeting a lesion for biopsy. Application of perfusion imaging may be particularly applicable to the study of neovascularization and angiogenesis inhibition.

### Activation Functional Magnetic Resonance Imaging

After the use of a stimulation paradigm or task designed to activate a specific functional area of the brain, the target can be located anatomically by an increase in blood flow to that area. Functional MRI uses the BOLD (*b*lood *o*xygen *l*evel–*d*ependent) technique to generate differential signals based on the relative hemoglobin-oxyhemoglobin content of the blood flowing away from activated brain during an appropriate stimulus. Repetition of the task improves the robustness of the data, and subtraction of rest from activity reduces background signal. Data are presented on maps that outline the activated area of interest in relation to the lesion, and these may be useful in preoperative planning. An example involving finger-thumb opposition and the motor cortex of the hand is shown in Figure 27-4. Different activation tasks can be designed to stimulate other eloquent areas of the brain for vision, hearing, and language.

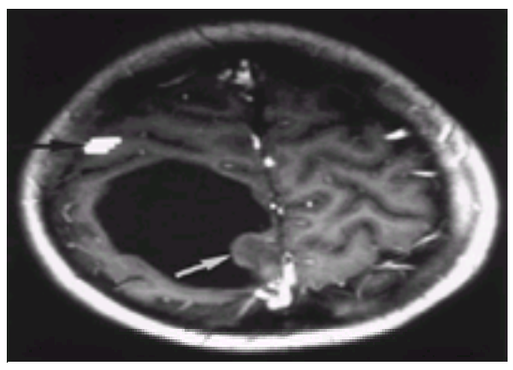


**FIGURE 27-4.** Preoperative functional magnetic resonance imaging in an 11-year-old boy presenting with left-sided focal motor seizures, performed for surgical planning. The bright pixels (*black arrow*) represent the statistical parametric map produced by using a left-sided finger-thumb opposition paradigm. The bright pixels reflect the increase in oxygenated blood flowing away from the right-sided sensorimotor cortex. The white arrow indicates the solid tumor component within a cystic mass displacing the sensorimotor cortex anteriorly. Histopathology demonstrated a cystic dysplastic ganglioglioma that later was removed successfully via a posterior approach without damage to the child.

### Single-Photon Emission Computed Tomography and Positron Emission Tomography

A limited number of reports address the role of thallium 201 single-photon emission CT (SPECT) in pediatric brain tumors. In a heterogeneous and somewhat limited series, although representative of the pediatric population, Rollins et al. [156] failed to establish any clinically useful indication for the test. $^{201}$Tl SPECT was less sensitive and less specific than was gadolinium-enhanced MRI and did not correlate with histologic grade, biologic aggressiveness, or tumor type. In children with brainstem glioma, however, $^{201}$Tl SPECT was able to distinguish persistent or recurrent tumor from radiation necrosis. [157]

Positron emission tomography (PET) adds another dimension to brain imaging. Using the appropriate tracer or combination of tracers, subtle metabolic changes can be measured with a relatively high spatial resolution approximating 3 to 4 mm. Registration of PET with MRI is an issue that will have to be resolved if meaningful

application of this technique using fluorodeoxyglucose and other isotopes is to occur in the clinical arena. [158]

## Imaging Characteristics of Central Nervous System Tumors

### *Posterior Fossa Tumors*

The differential diagnosis of cerebellar tumors in children consists in large part of medulloblastoma, or PNET, juvenile pilocytic astrocytoma (JPA), ependymoma, hemangioblastoma, and exophytic brainstem glioma. Although medulloblastoma tends to be more solid and homogeneously less enhancing and JPA more commonly cystic and typically strongly enhancing, distinguishing one from the other still can be difficult with CT or conventional MRI alone. However, DWI may help in the differential diagnosis preoperatively. Because of the high, tightly packed cellular content of medulloblastoma, a pattern of “restricted” diffusion appears to occur in these tumors, similar to that seen in stroke.[159] This is reflected by a reduction in the ADC. The ADC reflects physical factors, such as temperature and viscosity, in addition to the relative ease or restriction of the motion of molecules through tissues and membranes. The ADC of regions of tumors appears to correlate with cellularity, there being a tendency for lower ADC values to be seen with high-grade gliomas and higher values with low-grade gliomas. In comparing tumor tissue to normal brain, ADC may be important in distinguishing regions of edema from nonenhancing tumor as well. [160]

MRS may help also in the preoperative differential diagnosis of posterior fossa tumors. Using a discriminant analysis, single-voxel MRS of pediatric cerebellar tumors has been proven capable of separating out medulloblastoma (or PNET), JPA, and ependymoma from normal cerebellar tissue based on a plot of Cr/Cho ratios against NAA-choline ratios (Fig. 27-5).[161]

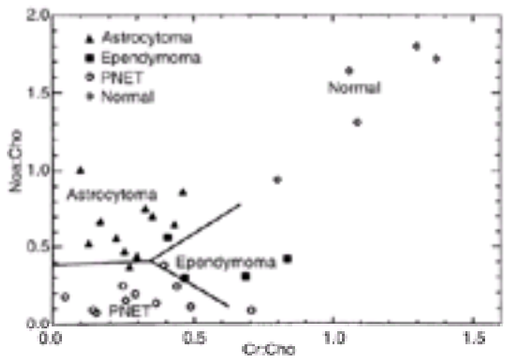



**FIGURE 27-5.** *N*-acetyl aspartate–choline (Naa:Cho) versus creatine-choline (Cr:Cho) scattergram for astrocytoma, ependymoma, medulloblastoma, and normal cerebellar tissue. The straight lines are boundaries between the three tumor types found by discriminant analysis. PNET, primitive neuroectodermal tumor.

Using CT, MRI, and MRS characteristics, the following can be said of posterior fossa tumors: The solid component of *JPA* is hyperdense on CT, hypointense to gray matter on T1WI, and hyperintense on T2WI, demonstrating marked enhancement after administration of gadolinium. DWI usually shows evidence of unrestricted diffusion, especially in the presence of a cystic component. MRS tends to show moderate reduction in NAA and modest elevation in Cho ( Fig. 27-6, Fig. 27-7). *Medulloblastoma* is hyperdense on CT and uniformly hypointense to gray matter on T1WI and tends to be isointense to gray matter on T2WI with restricted DWI, most likely secondary to the tumor's dense cellularity and high cellular nucleus-cytoplasm ratio. MRS tends to show very low NAA with very high Cho ( Fig. 27-8). *Ependymoma* may be distinguished by its different anatomic location and pattern of spread through the foramina of Luschka, but MRS can give additional support to the diagnosis, especially in those cases in which the size and extent of the mass render difficult the identification of its point of origin. Solitary cerebellar *hemangioblastoma* usually will demonstrate serpiginous flow voids, reflecting the vascularity of the lesion, in addition to intense enhancement after intravenous contrast.[162] Intrinsic hemangioblastoma may be seen also in the spinal cord.

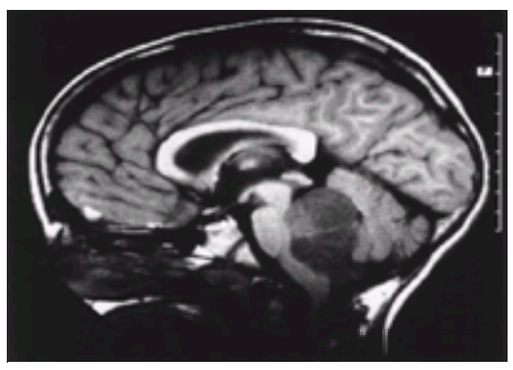


**FIGURE 27-6.** Sagittal T1-weighted midline image demonstrating posterior fossa tumor occupying most of the fourth ventricle extending inferiorly to the obex, with mild prominence of the supratentorial ventricular system. The histopathologic diagnosis was a juvenile pilocytic astrocytoma.

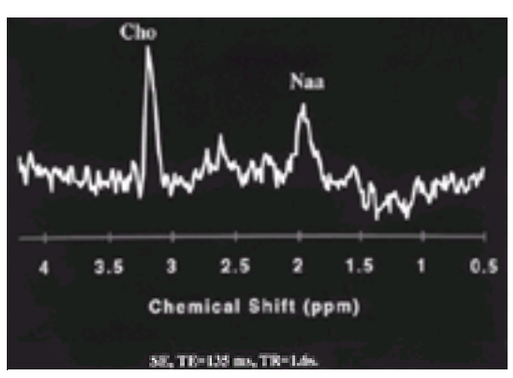



**FIGURE 27-7.** Long echo time (TE) magnetic resonance spectroscopy (135 milliseconds) from the same patient mentioned in Figure 27-6. Note the moderate decrease in *N*-acetyl aspartate (Naa) and moderate elevation in choline (Cho), a typical pattern for low-grade glioma.

Other rare tumors seen in the posterior fossa include AT/RT that are typically rapidly progressive, with surrounding edema and enhancement from breakdown of the BBB.

### *Brainstem Tumors*

MRI is the procedure of choice for identifying brainstem tumors. Some work has shown a correlation between $^{201}$Tl SPECT and gadolinium enhancement, suggesting that, like gadolinium, thallium uptake requires breakdown of the BBB. [157] The only information lacking with MRI is the presence or absence of calcification. Calcification is an unusual manifestation of brainstem tumors. It may occur rarely with oligodendroglioma of the brainstem, occasionally with extension of fibrillary astrocytoma of

the cervical cord into the medulla, or with dystrophic calcification secondary to radiotherapy. CT is superior at demonstrating calcification in these situations and in association with vascular lesions as well.

### *Supratentorial Tumors*

Intra-axial tumors, which usually are low-grade gliomas, are imaged in much the same way as are posterior fossa tumors. Published pediatric data concerning MRS of these lesions are few, but the peak-area ratios or NAA/Cho and Cr/Cho may be prognostic.[163] Experience in this area of imaging is evolving.

Ganglioglioma, DNET, and hypothalamic hamartoma have fairly specific imaging characteristics. Ganglioglioma and DNET tend to be superficial lesions with a predilection for the temporal lobe. Gangliogliomas may be cystic and demonstrate a variable pattern of relatively poor enhancement. DNET classically causes remodeling of the overlying inner table of the skull, a reflection of their natural history of slow growth. The anatomic location, lack of enhancement, and commonly associated history of gelastic seizures, precocious puberty, or other hormonal imbalance in a young child leads to a diagnosis of hypothalamic hamartoma.

Extra-axial sellar and pineal region tumors, together with meningiomas, lack a BBB and therefore enhance avidly after contrast administration. Single-voxel MRS has been used to differentiate large cystic sellar and parasellar masses on the basis of their metabolites.[164] Hypothalamic gliomas have a metabolic profile similar to that of JPAs and other low-grade gliomas, with a moderate reduction in NAA along with a moderate elevation in Cho. This is fairly different from pituitary adenomas, which demonstrate no NAA because they contain no neuronal tissue, a moderate elevation of Cho as a marker of cell membrane turnover, and variable amounts of lipid-lactate, depending on the degree of tissue necrosis. The “crankcase” oily contents of cystic craniopharyngiomas lead to a large lipid peak with little else.

### *Spinal Tumors*

Intrinsic tumors of the spinal cord, such as primary gliomas and ependymomas, are best imaged with MRI. It is also the imaging modality of choice for metastatic disease in the subarachnoid space (e.g., from medulloblastoma). Ideally, time and patient condition permitting, the spine is scanned preoperatively when medulloblastoma or ependymoma is suspected. When such a scan cannot be performed, a baseline study should be performed approximately 3 weeks into the postoperative period to avoid the problems of interpreting the appearances of postsurgical blood and arachnoiditis. Most treatment protocols direct repeat imaging for studies showing and not showing disease. However, it may be reasonable in the nonprotocol setting, when initial spine study results are negative, to repeat the spine MRI after completion of therapy only if new symptoms develop. CSF cytology is complementary to spinal imaging in detecting subarachnoid disease.

## Imaging Characteristics of Neurofibromatosis Type 1 and Associated Tumors

NF-1 frequently manifests as focal areas of signal intensity (FASI) on T2WI. Typical imaging features in affected young children include T2 bright signal returned from the basal ganglia and dentate nuclei, which gradually disappears in young adulthood. Limited histopathology suggests that these lesions may represent spongiform dysplasia.[165] However, it is recognized that some of these FASI grow and start to enhance and are diagnosed ultimately as gliomas, usually of low grade. Tumors identified in patients with NF-1 most commonly are located in the brainstem, optic chiasm, and hypothalamus and, less commonly, below the tentorium. Anomalies of the corpus callosum, including LGAs, have been identified also in children with NF-1.[166] Three-dimensional multivoxel proton MRS has been used in children with NF-1 to interrogate these areas of focal signal abnormality. Proton MRS indicated that FASI (a) are characterized by significantly elevated Cho, reduced Cr, a Cho/Cr ratio greater than 1.3, and near-normal NAA levels; (b) are different from tumors that exhibit Cho/Cr greater than 2 and no NAA; (c) have no lipid or lactate signal; and (d) correlate in spatial extent but are more extensive than indicated by conventional MRI sequences.[167] Use of three-dimensional chemical shift imaging, as opposed to single-voxel imaging spectroscopy, to delineate metabolically the extent and volume of the lesion, may be useful for following the effects of treatment ( Fig. 27-9).

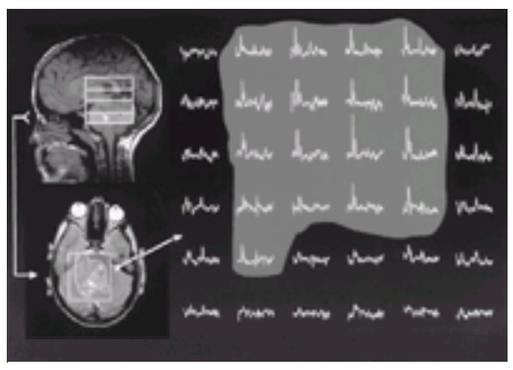


**FIGURE 27-9.** Composite image demonstrating a set-up for three-dimensional chemical shift imaging (stack of two-dimensional slabs), in a patient with neurofibromatosis type 1 complicated by a brainstem glioma. The tumor is outlined from the spectroscopy grid by the extent of the abnormal *N*-acetyl aspartate–choline pattern.

Screening neuroimaging of asymptomatic children with NF-1 has not been shown to improve clinical outcome. Serial ophthalmologic examinations of affected children are critical. Those with unexplained ophthalmologic abnormalities should undergo MRI examination of the head and orbits.[168] Optic nerve lesions seen in association with NF-1 generally are JPAs; they demonstrate enhancement on postgadolinium imaging. Unless directed otherwise by clinical protocol, follow-up imaging of these tumors should be considered only on evidence of deterioration of vision.

### *Tuberous Sclerosis*

In tuberous sclerosis, subependymal giant-cell astrocytoma classically occurs at the level of the foramen of Monro. These lesions often are bilateral, may be calcified, and usually demonstrate marked enhancement with intravenous contrast. They require continued follow-up and repeat imaging, on an annual basis, to track the development of hydrocephalus. Tubers typically demonstrate elevated myoinositol on MRS.

## Radiation Necrosis

Radiation necrosis may demonstrate mass effect, enhance after intravenous contrast, and easily be indistinguishable from recurrent or progressive tumor. Fluorodeoxyglucose PET has been considered the gold standard through which tumor and irradiation-induced necrosis can be distinguished. However, an incidence of false-negative and false-positive results now is recognized.[169] Two-dimensional chemical shift imaging/MRS can be used to interrogate the region of interest on a subcentimeter voxel-by-voxel basis. A reduction or absence of both NAA and Cho, in the presence of lipid and lactate, is found in radiation necrosis. In the presence of recurrent tumor, elevation of Cho occurs.

## Imaging and Radiation Treatment Planning

Many of the techniques described in this section allow for more accurate localization of tumor and tumor extent than was historically obtained with CT imaging. MRI should delineate more clearly between tissue planes and improve image fusion needed for planning radiotherapy. More experience is needed to determine whether this capability will result in improved patient outcome.

## Future Developments

Currently under development is the technique of whole-brain spectroscopy.[153] This modality is performed in conjunction with three-dimensional volumetric imaging of the brain and allows for a quantitative expression of individual metabolites, such as NAA and Cho, expressed per gram of brain tissue. Other advances in MRS include the development of two-dimensional double-quantum spectroscopy, which will further and more accurately separate out the metabolic peaks.[170,171 and 172]

Diffusion tensor imaging is a technique performed using six to nine planes of imaging as opposed to the three that typically are employed for DWI in standard MRI. The data acquired allow the mapping of white-matter tracts and detection of interruptions in them due to infiltrating tumor. [169] The same data can be used to measure fractional anisotropy, and those results may allow further discrimination of differential tissue characteristics.

Finally, the development of pulsed and continuous arterial spin tag labeling as a technique to study perfusion of brain tissue should permit quantitative or semiquantitative analyses of blood flow.[173] This procedure might have application to the assessment of angiogenesis in tumors and effects of anti-angiogenic therapy.

## NEUROSURGERY: DIAGNOSIS AND TREATMENT

For most CNS tumors, surgical intervention forms the initial step in the treatment plan by providing tissue to establish the histologic diagnosis and, when possible, reducing the tumor burden. Notable exceptions to this rule are certain unresectable tumor types, such as diffuse infiltrative brainstem gliomas [174] and globular chiasmatic gliomas in children with NF-1. Each of these tumors has an almost diagnostic MRI appearance in association with consistent histologic features. As surgery has been shown not to improve prognosis in patients with these tumors and the histologic diagnosis rarely is in question, operative intervention is not required. In addition, for certain deep-seated tumors, the risks imposed by a conventional surgical approach to them may be high and, for establishing a histologic diagnosis, stereotactic biopsy techniques provide a reasonable alternative to open tumor debulking. CT- or MRI-guided stereotactic procedures are highly accurate in targeting such deep-seated lesions and are associated with a morbidity of fewer than 5% and a mortality of fewer than 1% of patients. [175,176]

For the majority of pediatric brain tumors, however, open operations are preferred; the goal in such procedures is to remove as much tumor as is safely possible. Although a truly complete tumor resection is feasible only for well-circumscribed benign tumors, such as pilocytic astrocytomas and craniopharyngiomas, an extensive, near-total resection can be achieved with many parenchymal tumors. The limitation to complete resection in such tumors is the imperceptible blending of the neoplasm into the surrounding brain; scattered tumor cells may infiltrate past the margins of the resection into the normal parenchyma. Unlike the situation with solid, non-CNS tumors, it is rarely feasible to resect the tumor with surrounding margins of normal tissue because of the unacceptable risks of producing irreversible neurologic deficits. An outline of the neurosurgical approach and considerations for the most common types of brain tumors is provided in Table 27-6.

**TABLE 27-6. MANAGEMENT SCHEMA FOR PEDIATRIC BRAIN TUMORS[a]**

### Preoperative and Perioperative Considerations

In occasional affected children who present with obtundation from a large mass lesion, resection is performed urgently. In children who are awake and alert but nonetheless harbor a large lesion that is producing substantial mass effect, the tumor resection is performed on the next operating day. Smaller lesions without significant mass effect can be managed more electively.

Because peritumoral edema commonly contributes to the neurologic impairment produced by the tumor, moderate doses of corticosteroids generally are administered preoperatively. For example, 0.1 to 0.5 mg per kg dexamethasone may be given every 6 hours. This dosing often will lead to a dramatic improvement in the patient's symptoms and signs, avoiding the need for emergency surgery in the vast majority of cases. Corticosteroids typically are continued intraoperatively and during the early postoperative period. If a significant reduction in tumor volume has been achieved at the time of operation, corticosteroid therapy is tapered and then discontinued within several days of surgery.

Another factor that commonly contributes to increased ICP in children with brain tumors is the presence of obstructive hydrocephalus, observed most commonly in tumors arising near the aqueduct, such as with pineal region tumors, or the fourth ventricle, as seen with cerebellar vermian lesions. Although resection of the tumor often opens the CSF pathways and leads to resolution of the hydrocephalus, the resection frequently is rendered safer if the elevated pressure is relieved as an initial step. The use of preoperative shunting carries a small risk of upward herniation through the tentorial hiatus. In some institutions, preoperative relief of hydrocephalus is accomplished by endoscopic third ventriculostomy[177,178]; however, a more common approach is to place an external ventricular drain immediately before the craniotomy for tumor resection, which has the advantage of allowing drainage of bloody spinal fluid and debris in the early postoperative period. If the operative procedure opens the CSF pathways and the patient's absorptive pathways remain patent, the external ventricular drain often can be removed within several days of surgery.[179] If the hydrocephalus persists, a third ventriculostomy can be performed or, if the hydrocephalus appears to be of a communicating type, a ventriculoperitoneal shunt can then be inserted. Although in the past a great concern was that shunting would provide a route for systemic dissemination of tumor, more recent studies have failed to show an increased risk of tumor spread via the shunt. [180] Overall, the use of shunts in children with posterior fossa tumors has diminished substantially.

The endocrinopathies commonly manifested by hypothalamic tumors can be exacerbated by tumor resection. Patients undergoing this procedure typically require stress doses of hydroxycorticosteroids before, during, and after surgical intervention. Although thyroid hormone replacement occasionally is instituted preoperatively, it is initiated most commonly postoperatively. Patients in whom the posterior pituitary stalk is sectioned or injured during surgery often manifest a triphasic response of impaired fluid regulation, characterized by an initial period of transient diabetes insipidus lasting 1 to 2 days, a subsequent period of inappropriate antidiuretic hormone release lasting several days, and a final phase of persistent diabetes insipidus. In view of the rapid changes in vasopressin levels during the first several days postoperatively, careful attention to fluid replacement and cautious administration of synthetic vasopressin, where indicated, are essential to avoid potentially deleterious swings in electrolyte levels and fluid balance.

Children with cerebral cortical tumors and those in whom cortical retraction is required in the approach to a deep-seated lesion may be at risk for seizures during the perioperative period. Preoperatively, such patients often are started on an anticonvulsant medication (e.g., phenytoin) that is continued during the postoperative period, even if they have not experienced previous seizures. Patients generally are maintained on anticonvulsants for at least 1 week postoperatively; the decision to continue such therapy in patients without documented seizures is of uncertain benefit. [181] In patients who experience a preoperative seizure disorder from the tumor and have been rendered seizure-free by tumor resection, anticonvulsants often can be stopped within several months after surgery.

### Intraoperative Considerations and Surgical Technique

Recent studies have indicated that the extent of surgical resection has a major impact on the likelihood of long-term survival for children with many types of pediatric brain tumors, particularly ependymomas,[182,183,184,185,186,187 and 188] high-grade gliomas,[189,190,191 and 192] medulloblastomas,[193,194,195,196,197,198 and 199] low-grade gliomas,[200] and choroid plexus tumors.[201,202 and 203] Accordingly, with the exceptions noted earlier, in which surgical resection is not indicated or in which stereotactic biopsy may be a preferable initial step, extensive resection is the goal for many types of pediatric brain tumors.

A major limitation to the widespread incorporation of extensive surgical resections in the management of childhood brain tumors has been the fact that aggressive resections may increase the risk of immediate and long-term morbidity, particularly for tumors in functionally critical locations. Although morbidity generally is less than 10% for polar supratentorial gliomas and less than 20% for cerebellar astrocytomas, more deep-seated lesions, such as ependymomas and craniopharyngiomas,

carry morbidity rates in excess of 40% with extensive resection.[204] Although some studies have observed that morbidity is lower if operations are performed by neurosurgeons who do such operations frequently,[205,206] recent studies also indicate that pediatric neurosurgeons are more likely to attempt extensive removals, on the basis of their recognition that this influences prognosis, and therefore may have overall rates of management morbidity comparable to those of general neurosurgeons, albeit with a higher frequency of complete or nearly complete tumor resections.[207]

During the last 10 to 20 years, a number of intraoperative modalities have been developed or refined to allow tumor resection to be performed more safely and efficiently. Foremost among these are the progressive improvements in operative microscopy, which facilitates illumination and visualization of the interface between neoplasm and normal brain. Localization techniques, such as frame-based and, more recently, frameless stereotactic guidance systems, allow preoperative targeting of the tumor so that the surgical approach can be tailored precisely to minimize manipulation of normal brain structures and to maximize the extent of resection of deep-seated subcortical lesions.[208,209]

Ultrasonographic guidance also is useful in this regard. Recently, intraoperative MRI units have become available in selected centers and may help to refine further the accuracy of intraoperative decision making,[210] provided that issues of cost and the difficulties involved in operating in or adjacent to a high field-strength magnet can be resolved effectively.

In children whose tumors are in and around functionally critical brain regions, intraoperative monitoring of visual, auditory, and somatosensory pathways and direct assessment of motor and speech pathways often are used in an attempt to improve the safety of the tumor resection. In addition, areas of essential cortex overlying a deep-seated tumor may be delineated using cortical stimulation techniques to plan an approach to the tumor that avoids traversing important structures. Functional MRI also provides a useful way of noninvasively localizing important cortical areas[211] to identify a safe trajectory to an underlying lesion. Finally, in children with intractable seizures from cerebral neoplasms, intraoperative or extraoperative electrocorticography (ECOG) may be used to define areas of epileptogenic cortex in and around the tumor to increase the likelihood that seizure control will be obtained postoperatively.[212]

For supratentorial craniotomies, children generally are placed in the supine or lateral position. For infratentorial craniotomies, the prone or lateral position is used more often than the upright position because of concern over potential venous air embolism. Intraoperatively, the head of an infant often is positioned on soft rings rather than being held by pins that can perforate the skull or cause a depressed fracture. For cortical and many subcortical tumors, the surgical approach follows the most direct trajectory to the lesion. However, for deep-seated lesions that are subjacent to functionally critical regions of the brain, alternate approaches often are required. Details of the operative approaches for specific tumor types are provided in subsequent sections of this chapter.

The actual tumor resection often is aided by the use of ultrasonic aspiration, which provides a relatively atraumatic way to debulk many pediatric brain tumors. The surgical $CO_2$ laser also may be used, depending on the consistency and location of the tumor. In general, tumors are resected “from the inside out.” With many extra-axial tumors and a small percentage of intraparenchymal lesions, a clearly defined peritumoral plane is encountered through which the tumor may be dissected carefully from the surrounding brain, cranial nerves, and vessels after the central portion of the mass has been debulked. However, for most intraparenchymal tumors, a well-defined tumor pseudocapsule is not present, and the resection must proceed via gradual internal debulking until the boundary between tumor and normal brain is reached.

Because the extent of resection is so important in defining prognosis and choice of subsequent therapy for many tumor types, objective confirmation of the volume of residual tumor, if any, is essential before embarking on further therapy. Because a surgeon's impression of the extent of tumor resection is subject to error,[213] postoperative confirmation of the extent of resection generally is established by CT or, preferably, by MRI. This imaging typically is performed within the first 24 to 72 hours postoperatively to minimize the impact of postoperative inflammation on the delineation of areas of residual tumor.

A recent trend in the surgical management of selected types of brain tumors has been the concept of second-look surgery. For large, relatively vascular tumors in which an initial complete resection cannot be obtained, the patient is treated with several courses of postoperative chemotherapy in the hope of making the tumor amenable to complete resection at a second procedure. This approach has been applied anecdotally in ependymomas and malignant germ cell tumors, two groups of lesions in which the extent of residual disease before initiation of radiotherapy has a substantial impact on long-term outcome. What remains to be determined is whether patients who undergo a second-stage complete resection have as good a prognosis as those who were amenable to complete resection initially; this issue is being examined systematically in studies of the Children's Oncology Group (COG).

Surgical resection has been used increasingly as a component of the management of recurrent disease, particularly in children without evidence of tumor dissemination. For children with malignant lesions, this relieves mass effect in preparation for additional phase I or phase II chemotherapy. Some recurrent tumors, such as JPAs and craniopharyngiomas, can be treated with reoperation alone, without the need for additional adjuvant therapy, if a gross total resection (GTR) can be achieved.[214] For children with recurrence of other, more malignant tumors that may also be subject to dissemination, data are lacking for a survival benefit from re-resection.

## RADIOTHERAPY

The rational application of radiotherapy in pediatric brain tumors requires an understanding of the development of the brain, the probable biologic effect of ionizing radiation on the brain of a child, the behavior and natural history of the different brain tumors, radiobiology and physics, the techniques and technology of radiotherapy, and the probable interaction of irradiation with other treatment modalities, such as chemotherapy.

### Radiation and the Developing Brain

The development of brain is most rapid during the first 3 years of life. Axonal growth and synaptogenesis are most active during the growth phase.[215] The rate of growth and development slows down after the age of 6. Maturation of the brain, however, judged by degree of myelinization, is not complete until puberty.[216] Hence, radiation-induced brain injury is most pronounced during the early years of childhood. Radiation injury to the brain generally is regarded as one of the most serious complications of radiotherapy of brain tumors and thus constitutes the major limitation in delivering high-dose radiation.

### Effect of Radiation on the Brain

The responses of brain tissues to radiation are classified into three groups: (a) acute reactions, occurring during treatment; (b) early delayed (subacute) reactions, occurring a few weeks to 2 to 3 months after treatment; and (c) late delayed reactions, occurring several months to years after treatment.[217] The pathogenesis of radiation-induced brain injury includes edema formation; damage to oligodendrogliocytes, leading to inhibition of myelin synthesis; and damage to the vascular endothelium, leading to white-matter necrosis. The late delayed reactions, which include focal radiation necrosis, postirradiation diffuse white-matter injury, leukoencephalopathy, neuropsychological effects, cerebrovascular effects, and secondary tumors, can be progressive, irreversible, and potentially fatal. Late neuropsychological effects include intellectual impairment, memory deficits, and inability to acquire new knowledge. Impairment in cognition is most pronounced in children younger than age 4 to 7. Deterioration in intelligence quotient (IQ) is prevalent in children after whole-brain or supratentorial irradiation for primary brain tumors.

The presence and severity of radiation reactions depend on (a) irradiation treatment factors, including total dose, fraction size, interfractional interval, and treatment volume; (b) patient factors, such as age, presence of preexisting brain injury by tumor or surgery, infection, and vascular diseases; and (c) other treatment modalities, most commonly surgery and chemotherapy. Some factors are unavoidable, such as preexisting brain injury or vascular diseases. However, the influence of certain factors, such as fraction size and treatment volume, can be altered to decrease the incidence and severity of brain injury. One of the latest advances in radiation oncology is conformal radiotherapy, which certainly can decrease the irradiated volume and potentially minimize treatment-related toxicity.

### Radiosensitivity of Specific Structures in the Central Nervous System

#### *Brainstem*

The brainstem traditionally was regarded as more radiosensitive than the cerebrum, especially in the era of orthovoltage. However, in the modern era of high-energy radiotherapy, brainstem necrosis is rare. In view of the high concentration of white matter, a reduction of 10% from brain tolerance dose usually is recommended.[218]

Fraction size also is very important. Other influencing factors include chemotherapy and vascular pathology.

### *Spinal Cord*

In view of its location, the tolerance of spinal cord to radiation is a major dose-limiting factor in delivering high-dose radiation not only to tumors involving the cord but to other tumors in the vicinity. In pediatric radiation oncology, craniospinal irradiation is common, especially in the treatment of standard- and high-risk medulloblastoma. Also, in irradiating certain head and neck tumors in childhood, such as rhabdomyosarcoma and nasopharyngeal angiofibroma, the spinal cord usually will receive some radiation. If the spinal cord is overdosed, damage can result in devastating neurologic deficits. Radiation myelopathy can occur from 1 year to several years after cure of the cancer.[219] Instead of tumor cure, quality of life then becomes a very important issue. The traditional dogma concerning the pathogenesis of radiation myelopathy rests on postmitotic cell death in the endothelial cells or oligodendrocytes (or both). The current concepts view radiation as producing cell death that, in turn, induces a complex pathophysiologic reaction in which the response of surviving cells may contribute to determining the impact of radiation on tissue integrity and functions. Cytokines, such as tumor necrosis factor and interleukin-6 (IL-6), appear to play important roles. [219] Also, some researchers suggest that the tolerance of the spinal cord is 5% to 10% lower in children than in adults. [219] Increasing the radiation dose per fraction generally produces a disproportionately larger biological effect on the spinal cord. If the time interval between fractions is less than 8 hours, the repair by the spinal cord of sublethal damage could be incomplete and consequently the biological effect could be magnified. Interfractional interval also plays an important role in radiation myelopathy. Generally accepted is that the dose to the spinal cord should be reduced when the irradiated volume is large. Results of recent primate studies indicated that an increase in treatment volume reduces the threshold and steepens the slope of the sigmoid dose-response curve for myelopathy. [219]

### *Cranial Nerves*

Most cranial nerves are relatively resistant to radiation-induced damage. Two cranial nerves, the second and eighth, are worth mentioning in the radiation treatment of pediatric cancers. The optic nerve and visual pathway can be damaged when delivering therapeutic radiation to periorbital tumors (e.g., orbital rhabdomyosarcoma, optic glioma, paranasal and nasopharyngeal tumors) and suprasellar tumors (e.g., craniopharyngioma, pituitary adenoma, germ cell tumor, hypothalamic-chiasmatic glioma). Recently, a linear-quadratic model yielded an a/b estimate of 1.6 Gy for optic neuropathy. [220] The risk of radiation-induced optic neuropathy is related to total radiation dose, fraction size, and the irradiated volume. It was shown recently that no injuries were observed in 106 optic nerves that received a total dose of less than 59 Gy. The 15-year actuarial risk of optic neuropathy after a dose of greater than 60 Gy was 11% when treatment was administered in fraction sizes of less than 1.9 Gy, as compared with 47% when given in fraction sizes of greater than 1.9 Gy. [221] The vestibular cochlear nerve and auditory apparatus must be considered in the delivery of high-dose radiation to tumors in the posterior fossa, such as medulloblastoma, ependymoma, and astrocytoma. Medulloblastoma therapy often includes cisplatin-based chemotherapy. Cisplatin given after completion of radiotherapy increases further the risk of ototoxicity.

### *Retina*

The retina, a specialized neural end-organ supplied by an end-arterial system, is sensitive to vascular injury and has little ability for repair. [217] It is sensitive to radiation as well. Deterioration of vision, resulting from radiation-induced progressive obliteration of small retinal vessels, can occur 1.5 to 6.0 years after irradiation. The dose-response curve is steep (between 50 and 60 Gy), and 45 Gy produces a 5% risk of visual injury within 5 years. [218] Again, as the fraction size increases up to 2.5 Gy or more, the frequency of injury increases.[222]

### *Lens*

The lens is one of the most radiosensitive organs, even to very low doses of radiation. For example, 1 Gy can lead to cataract formation. From total body irradiation data, the risk of developing a cataract requiring surgery was 20% for fractionated doses of 12 to 16 Gy. [223] Currently, the dose that could produce a 5% risk of damage to the lens within 5 years is 10 Gy.

### *Hypothalamic-Pituitary Axis*

Irradiation of the region of the hypothalamus and pituitary gland can result in significant neuroendocrine abnormalities and long-term sequelae. This is especially important in children. The hormones affected include growth hormone (GH), thyroid-stimulating hormone, adrenocorticotropic hormone, and follicle-stimulating hormone–luteinizing hormone. The largest volume of data concerns the effect of cranial irradiation on GH production and release. The irradiated anatomic site responsible for GH deficiency has been shown to be the hypothalamus.[224] Sixty percent to 80% of irradiated pediatric brain tumor patients ultimately will have impaired serum GH response to provocative stimulation.[224] A dose-response relationship is seen with a threshold of 18 to 25 Gy. The higher the dose of radiation, the earlier the GH deficiency will occur. Deficiencies of other hypothalamic-pituitary hormones also have been described. The responsible irradiated site can be the hypothalamus, the pituitary, or both. Constine et al.[225] have described non-GH abnormalities (thyroidal, gonadal, prolactin, and adrenal) in 20 children with brain tumors not involving the hypothalamic-pituitary region and treated with either cranial or craniospinal irradiation. In patients receiving only cranial irradiation, the hypothalamic-pituitary region is estimated to receive a mean dose of 53.6 Gy (40 to 70 Gy). [225]

## Potential Side Effects of Radiotherapy

Acute and subacute toxicities related to radiation of the pediatric brain and head and neck are mild and usually produce no major consequences. Acute reactions, characterized by symptoms suggesting increased edema and ICP, or deterioration of preexisting neurologic signs, usually can be controlled and reversed by steroids. Subacute reactions, characterized by somnolence syndrome or accentuation of preexisting signs and symptoms, usually are transient and associated with complete recovery. Late sequelae are of major concern. They are discussed in detail in the section Sequelae of Treatment.

## Indications for Radiotherapy

The indications for radiotherapy depend on the histology of the tumor. Therefore, pretreatment histologic diagnosis is necessary in practically all cases. The only exceptions are diffuse pontine glioma and optic gliomas in which a diagnosis can be made with high certainty on the basis of neuroimaging. Specific indications for radiotherapy and controversies concerning its use are discussed later under the headings of the individual tumor types.

## Radiotherapy Volume

Radiotherapy volume is determined by the anatomic extent of the tumor and the potential areas of spread and by the patterns of failure. Advances in MRI and CT have made tumor localization more accurate.

In general, local target volumes are used for tumors that are either confined to, or recur within, a single anatomic area. Whole-brain irradiation is used for multifocal tumors. Craniospinal irradiation is delivered to patients who have tumors with proven or suspected seeding in the subarachnoid space or to those with a reasonable chance of developing subarachnoid disease.

## Radiobiologic Considerations

The biology of fractionation in radiotherapy is based on four *R*s: reoxygenation, redistribution of cells within the cell cycle, repair of sublethal damage, and repopulation. These functions are discussed in Chapter 13.

The effect of radiation on the brain generally is characterized by delayed reactions, because the normal brain parenchymal cells are either slowly or not dividing. As a late-responding tissue, the normal CNS is very sensitive to radiation dose fraction size. Small radiation fraction sizes tend to produce a lesser effect on the CNS than on tumor cells. One can take advantage of this phenomenon by decreasing the fraction size from what is considered conventional and delivering more than one fraction each day. This is called *hyperfractionation*.

Hyperfractionated radiotherapy has several theoretic benefits: (a) the opportunity to escalate radiation dose without increasing damage to late-responding tissues; (b) greater ability to overcome tumor hypoxia that contributes to radioresistance; and (c) increased tumor cell kill by the dose escalation. Generally, a sufficient interval

between fractions is necessary to allow normal tissue repair of radiation-induced damage between the radiation fractions. Six to eight hours commonly is used for the interval. This approach has been tested in the treatment of supratentorial and brainstem high-grade glioma. However, to date, no convincing data suggest any survival benefits with the use of a hyperfractionated radiotherapy approach, probably because the total radiation dose still is insufficient to show a significant increase in tumor cell kill.

Accelerated fractionation radiotherapy is another fractionation technique. The aim with this approach is to deliver the total radiotherapy dose over a shortened period. It involves multiple daily radiotherapy fractions in conventional radiotherapy fraction size (usually 1.6 to 2.0 Gy). The total radiotherapy dose may be equivalent to or less than that delivered with conventional fractionation. Shortening of overall treatment time has the theoretic benefit of overcoming the accelerated repopulation of tumor clonogens between radiation fractions.[226] Again, no convincing data suggest a survival advantage with the use of accelerated fractionation over conventional radiotherapy.

### Techniques in Radiotherapy

#### *Conventional External-Beam Radiotherapy*

Conventional radiotherapeutic techniques remain the most common in use today. These techniques use simple geometric radiation-field arrangements with a small number of treatment fields. Head immobilization is important so that the treatment target is not missed during radiotherapy. A customized head-fixation device, such as an Aquaplast mask, frequently is used. Other immobilization devices, such as the body cast or alpha cradle, may be used when appropriate. Sedation or general anesthesia usually is necessary in the treatment of children younger than ages 3 or 4 years. The advantages of conventional techniques are relative simplicity (except perhaps for craniospinal irradiation) and general familiarity by radiation oncologists and technologists. The major disadvantage is the usual inclusion of more normal brain tissue than necessary in the high-radiation-dose regimen because of the limited field arrangements.

#### *Classic Three-Dimensional Conformal Radiotherapy*

The advances in neuroimaging and computers have rendered possible the delivery of high-dose radiation more precisely to the tumor while significantly sparing the surrounding normal tissues when using three-dimensional conformal radiotherapy (3D-CRT). This technique may involve multishaped fields, arranged in coplanar, nonaxial, and noncoplanar orientation, directed at the tumor, and delivered in either static or dynamic modes. [227] Compared to conventional radiotherapy, 3D-CRT results in the reception of high-dose radiation by approximately 30% less normal brain tissue. [228] This reduction in normal brain irradiation should lead to fewer long-term side effects, particularly in young children.

#### *Intensity-Modulated Radiotherapy*

Intensity-modulated radiotherapy (IMRT), a more complex form of 3D-CRT, combines two advanced concepts to 3D-CRT: (a) inverse treatment planning with optimization by computer and (b) computer-controlled intensity modulation of the radiation beam during treatment. IMRT allows a high degree of flexibility in reducing the dose to the surrounding normal tissues by the creation of so-called avoidance areas during the treatment planning process. The initial experience with IMRT in the treatment of brain tumors has been encouraging.

#### *Stereotactic Radiosurgery*

Radiosurgery can be administered by a gamma-knife unit using cobalt 201 sources or by a modified linear accelerator unit. Either unit is designed to deliver a high radiation dose to a small intracranial target in one sitting by focusing multiple small radiation beams from different directions to the target. The application of radiosurgery usually is limited to tumors measuring 3.5 cm or less in maximum diameter. This limit is held to achieve the steep dose gradient at the treatment-field edge. Radiosurgery commonly is used for the management of brain metastases, acoustic neuroma, and meningioma and is being investigated as a boost treatment for small, high-grade gliomas.

#### *Brachytherapy*

Brachytherapy, or interstitial irradiation, involves the implantation of radioactive sources directly into brain tumors. Iodine 125, iridium 192, and gold 198 are the radioisotopes that have been used for either permanent or temporary implants. Brachytherapy has been used for patients with glioblastoma as an additional boost after external-beam radiotherapy and for selected patients with recurrent high-grade glioma.

## PRINCIPLES OF CHEMOTHERAPY

Specific indications for the use of chemotherapy and the results of current trials for each tumor individually are discussed later. Those issues that relate to the use of chemotherapy for CNS tumors and an overview of new therapeutic approaches are presented here.

### Blood–Brain Barrier and Other Factors Influencing Drug Penetration

The presence of the BBB profoundly influences the penetration of most substances in the CNS. Tight endothelial cell junctions of this structure limit penetration to all but small-molecular-size (200 Da), highly lipophilic compounds, unionized at physiologic pH. [229] The BBB apparently is not uniformly intact in CNS tumors, however, and various degrees of disruption exist in different parts of these tumors. This variability is reflected in the different degrees of enhancement in CNS tumors produced by the water-soluble contrast agents commonly used with CT and MRI. In general, small tumor foci and the peripheral leading edges of larger tumors sometimes appear to have a relatively intact BBB. Furthermore, central portions of larger tumors may have absent BBB characteristics, independent of central necrosis. [230] These findings may be explained by the advancing margins of tumor that initially parasitize normal CNS capillaries that possess an intact BBB and by abnormal tumor-induced vascularity to arise in and dominate more established areas of tumor. [231]

These observations suggest that the commonly held notion that water-soluble chemotherapeutic agents are unlikely to be useful in CNS tumors is not entirely correct. In fact, many such compounds, such as the classic alkylators and the platinums, are of clinical value in these tumors. However, for a greater effect from chemotherapy at the peripheral, advancing areas of tumor in which the BBB is relatively intact, the use of more liposoluble agents may be required.

Other properties of individual agents that influence CNS tumor penetration include the degree to which they are protein-bound and the rapidity with which they are cleared systemically. Clinical variables affecting drug penetration include the use of corticosteroids, anticonvulsants, and concurrent radiotherapy. Steroids both may affect the systemic metabolism of agents and have been suggested to decrease the transcapillary transport of various compounds. Anticonvulsants may accelerate hepatic clearance of agents and, therefore, systemic and CSF levels of drug. [232] Radiotherapy at higher doses may increase transcapillary transport. [233,234] and [235]

### Drug Delivery Strategies

#### *High-Dose Systemic Therapy*

Tumors of the CNS may fail to respond to standard-dose chemotherapy because of inherent or acquired drug resistance. Additionally, treatment may fail because of a relative lack of penetration of agents into the CNS and further into the tumor tissue. In an attempt to overpower these resistance mechanisms, several clinical trials have examined the use of high-dose chemotherapy, with autologous bone marrow or peripheral blood stem cell rescue, to try to improve the response rate of CNS tumors to chemotherapy. Because of their liposolubility, the nitrosoureas were among the first agents so studied. However, because of excessive neurologic toxicity, dose escalation of these agents was not feasible. [236,237] and [238] More recent trials have used classic and nonclassic alkylating agents, often combined with etoposide. Examples of the former include cyclophosphamide, melphalan, and thiotepa; the nonclassic group includes carboplatin. The rationale for these drug combinations is based on their nonoverlapping hematologic toxicities, their propensity to show little cross-resistance, and their maintenance of steep, linear dose-response curves through several logs of cell kill. The approach has been studied most often in patients with recurrent tumors and in infants, for whom postponement of radiotherapy is desirable because of its potential late neurologic toxicities. [239,240,241,242,243,244] and [245] Feasibility has been demonstrated clearly, and encouraging results have been seen in patients with medulloblastoma and germ cell tumors and in infants with embryonal tumors. Clinical trials wherein these regimens are compared prospectively to

those using standard-dose chemotherapy have not been performed. The experience with gliomas, including those of the brainstem, has been less promising. [246,247] and [248] Patients who appear most likely to benefit from this approach are those with minimal disease at entry into myeloablative therapy, those whose tumors have shown response to standard-dose chemotherapy, and those with little prior exposure to chemotherapy. The optimal timing of this approach, either as a consolidative or a salvage treatment, remains uncertain, particularly as primary chemotherapy regimens are intensified. Because it is a feasible approach, high-dose chemotherapy with hematopoietic rescue will continue to be investigated for high-risk tumors. However, such treatment outside a clinical trial is inappropriate.

### *Intrathecal Chemotherapy*

Delivery of drug directly into the intrathecal space through either a lumbar puncture or a ventricular reservoir is a type of regional chemotherapy designed to circumvent limited penetration through the BBB of systemically administered agents. Compared to the result with plasma, the volume of CSF is relatively small and, as a result, high concentrations of drug can be achieved after low doses of intrathecal agents. However, penetration of drugs from the CSF into the parenchyma of the spinal cord and brain is limited severely by the efficient absorption of the compounds by the rich capillary vascular supply throughout the CNS. [249] Therefore, a benefit from intrathecal chemotherapy is likely to be realized only for control or prevention of subarachnoid disease. Mafosfamide and topotecan are two agents currently under investigation by clinical cooperative groups; busulfan will be studied by the Pediatric Brain Tumor Consortium (PBTC). Mafosfamide is the preactivated derivative of cyclophosphamide and has shown cytotoxic activity *in vitro* comparable to or exceeding that of cyclophosphamide and *in vivo* against numerous human solid tumor cell lines.[250,251] Topotecan is a water-soluble analog of camptothecin and an inhibitor of topoisomerase-1. Although the number of agents currently approved for intrathecal administration is limited, the potential utility of this route is being documented for several new agents, including those used in such new approaches as immunotherapy and gene therapy.[252]

### *Intratumoral Chemotherapy*

Another innovative strategy to enhance delivery of a therapeutic agent is direct administration of the agent into the tumor bed. Although this approach has been studied most extensively in adults with recurrent high-grade gliomas, it now is being translated to the pediatric setting. The most experience to date has been gained with interstitial chemotherapy, using polymeric “wafers” impregnated with BCNU (carmustine), which relies on passive diffusion of the nitrosourea from the polymer during a period of several weeks to achieve high drug concentrations around the tumor while keeping systemic exposure at low levels. Studies by Brem et al. [253] and Valtonen et al.[254] have demonstrated a modest prolongation of the survival of adults with recurrent high-grade gliomas, and a recent study of patients with newly diagnosed disease showed a similarly modest but nonetheless statistically significant therapeutic effect. [255]

An alternate approach, better suited for the delivery of larger molecules, involves the direct infusion of a soluble agent into the tumor using an implanted catheter connected to an external infusion pump. This approach incorporates the properties of bulk flow through the interstitial spaces of the brain to provide high concentrations of a therapeutic agent to the tumor and peritumoral brain. Although this approach has been applied anecdotally for the delivery of conventional chemotherapeutic agents, such as BCNU, the most experience to date has been obtained in the delivery of mutated toxin genes conjugated to ligands that target receptors (e.g., epidermal growth factor receptor, transferrin receptor, IL-13 receptor) expressed at levels higher within the brain tumor than in the surrounding normal brain.[256,257] The toxin component of the conjugate, such as diphtheria toxin and *Pseudomonas* exotoxin, contains mutations within the domains necessary for cell internalization, which restricts toxin entry to those cells that express the receptor for the ligand. Because each of the receptors that has been targeted to date is expressed also on cells outside the CNS, the infusion approach to delivery minimizes the concentrations of the toxin that reach those vulnerable non-CNS cells while maximizing the amounts that are delivered to the tumor.

### *Blood–Brain Barrier Disruption*

Osmotic opening of the BBB by infusions of hypertonic arabinose or mannitol can enhance the penetration of different compounds into the CNS. The mechanism appears to involve vasodilation, shrinkage of endothelial cells, and opening of endothelial tight junctions and results in an increased diffusion and bulk flow of water-soluble and large-molecular-size substances that normally are unable to permeate the BBB. [258,259] Although the effect is brief (generally reversible within 10 minutes), increases in CNS and CSF drug levels have been documented and correlated favorably with clinical responses in some instances. [260,261,262] and [263] Phase III studies are lacking, and one published experience showed no benefit from disruption of the BBB. [264] Pediatric experience is fairly limited, and only one study has evaluated the effect of mannitol infusion in a phase II study of an agent (etoposide). [265] In that study, overall response rate was approximately 11%, although it varied with tumor type, and an effect of mannitol infusion was not observed. The study could not determine whether the lack of effect was due to failure to disrupt the BBB or to a low level of activity of etoposide. A number of controversies surround this approach to therapy, including the contribution of the BBB to poor outcome of therapy and the clinical impact of the ability to circumvent this barrier. Furthermore, methodologic standards are lacking. Carefully designed clinical trials are needed to test this treatment approach.

### *Carotid Artery Infusion Chemotherapy*

The rationale for infusion of chemotherapy through the carotid artery is that lower doses of chemotherapy more immediately might be delivered directly to the region of the tumor, resulting in a higher concentration of drug in the tumor bed without a concomitant increase in systemic exposure and toxicity. Agents that penetrate tumor well but have a rapid systemic clearance theoretically are the best candidates for this approach, and increased regional exposure should be possible with lower doses of the agents. The use of nitrosoureas and cisplatin in this manner has resulted in a modest number of clinical responses. [238,266,267] However, drug penetration into normal brain tissue appears to have been increased as well, resulting in focal neurologic toxicity, particularly to the retina. [268,269] Some of the toxicity may have been due to nonuniform mixing of drug and blood at the infusion site, resulting in “streaming” during arterial delivery and to exposure of the brain to alcohol-containing diluents.[269,270]

## Other New Approaches

### *Differentiation Therapy*

The potential utility of differentiating agents, such as retinoic acid and phenylacetate, has received attention in human CNS tumors. Various *in vitro* studies have suggested that these compounds may induce differentiation and suppress growth in human CNS tumor lines, including glioblastoma multiforme and medulloblastoma.[271,272,273,274,275,276,277,278] and [279] The mechanism of these effects remains uncertain. Retinoic acid may modulate autocrine growth loops in some tumors; an inhibition of the kinase activity of epidermal growth factor receptor has been suggested by some investigators. [280] This molecule often is amplified in adult high-grade gliomas and possibly is related to malignant transformation. To date, phase I trials of both *trans*- and *cis*-retinoic acids have been reported preliminarily in adult glioma patients; an objective response rate of approximately 12% and a stable disease rate of 12% to 35% have been reported after oral administration in small numbers of patients.[281,282] and [283] The mechanism of action for phenylacetate has been hypothesized to involve DNA hypomethylation with secondary alterations in cycle-regulatory proteins. The demonstration of some modulation of *in vivo* proliferation and biochemical changes suggests differentiation in some malignant glioma and medulloblastoma cell lines.[274] An adult phase I study of this agent suggested that potentially therapeutic levels of phenylacetate may be achieved. [284] An oral formulation of phenylbutyrate has been studied in adults, also demonstrating that drug concentrations beyond the *in vitro* therapeutic threshold can be achieved.[285] Pediatric investigations are ongoing.

### *Immunotherapy*

The CNS is a relatively immunologically privileged site. The brain lacks defined lymphatic drainage, [286] the expression of major histocompatibility complex antigens is low,[287] and the BBB limits the interaction of the peripheral host immune system and the brain.[288] However, the privilege is not absolute. For example, patterns of allogeneic and xenogeneic tissue transplant rejection from immunologically naïve [289] and nonnaïve brains[290] suggest that peripheral T-cell activity may be carried into the CNS.[291]

Immunotherapy of CNS tumors is based on the hypothesis that stimulation of the immune system, or blocking of the immunosuppressive effects of tumors, might enhance an antitumor response. Immunotherapy has been studied primarily in the preclinical setting but also more recently in adult phase I studies of patients with malignant gliomas. Pediatric data are in their very early stages. Strategies of immunotherapy are based on eliciting systemic antitumor immune responses that are carried into the CNS and on inducing a primary immune response in the brain itself. Adults with malignant gliomas are known to have, to some degree, altered immunity, owing to effects on T-cell proliferation, natural killer cell activity, and immunoglobulin production. The current thought is that these effects most likely are

due to production of transforming growth factor b. These observations form the basis for another immunotherapy strategy, that of decreasing tumorigenicity of malignant gliomas by blocking the immunosuppressive effects of transforming growth factor b. A variety of approaches have been studied: administration of cytokines, such as ILs and interferons; delivery of monoclonal antibodies; and the use of adoptive immunotherapy (i.e., the transfer of immune T lymphocytes). Gene transfer therapy is discussed later.

Interferons show cytostatic and cytotoxic effects on human glioma cell lines and xenografts.[292,293 and 294] However, phase I and phase II clinical trials of interferon-a and interferon-b to boost systemic immune responses against intracranial tumors have yielded clinical responses in only a minority of patients.[295,296,297 and 298] In addition, clinical trials of interferon-g, which is a much more potent inducer of major histocompatibility complex class I and II antigen expression, have demonstrated unacceptable toxicities with little clinical benefit.[299,300] Although the interferons appear to have anti–CNS tumor properties, their clinical potential has not yet been realized.

IL-2, a cytokine that can increase the antitumor activity of T cells and natural killer cells, has been used in the treatment of melanoma and renal cell carcinoma. Systemic and intratumoral administration of IL-2 to boost the antitumor cellular immune response of patients with malignant gliomas has resulted in significant neurotoxicity, primarily from cerebral edema, and in little clinical benefit.[288] Adoptive immunotherapy using IL-2 and lymphokine-activated killer cells has resulted in inconsistent clinical and neurotoxic effects.[301,302] IL-2 and tumor-infiltrating lymphocytes appear to have activity against CNS tumors *in vitro* and in extracerebral sites but not in the brain.[303] In yet another adoptive approach, stable disease and prolonged survival, albeit with disease, were demonstrated in a recent adult phase I clinical trial of cytotoxic T-lymphocyte therapy for patients with primary or recurrent malignant gliomas.[304] Taken together, these data indicate an *in vitro* antitumor potential of adoptive immunotherapy of CNS tumors that has yet to be fully realized in the clinical setting.

A potential role for monoclonal antibodies in the diagnosis and treatment of brain tumors also has been investigated. Systemically administered radionuclide-conjugated antibodies have prolonged survival in mice with human glioma xenografts, but human applications of these products have shown only limited efficacy. Although disrupted by tumor, the BBB may be sufficiently intact as to block penetration of large-molecular-weight antibodies.[305] The application of these products currently is limited by tumor heterogeneity and rapid immune antibody clearance and, for radioconjugated antibody therapy, by dehalogenation and loss of radionuclides[131] and excessive radiation to nontarget tissues.[306] Intrathecal or intraventricular delivery of monoclonal products may bypass some of the limitations of systemic administration. Such studies are limited but promising.[307,308 and 309]

### *Gene Transfer Therapy*

Gene transfer therapy, the process through which genetic material is transferred into cells for the purpose of eliciting a therapeutic response, is a ew and innovative approach to the treatment of brain tumors and of other malignancies and disease processes (see Chapter 17). The gene of interest generally is transferred to the target brain or tumor cell using a virus-mediated delivery system. The postmitotic environment of the CNS tissue may offer an advantage over other tissues in that it may allow more specific targeting of the viral vectors to only mitotically active tumor. Genes that may be transferred may result in cell killing, either directly through cellular toxins or indirectly through the expression of drug-mediating enzymes.[310] The therapeutic response of gene therapy can be through immunomodulation or anti-angiogenesis as well.[291,310,311 and 312]

The herpes simplex virus thymidine kinase type 1 (*HSV-Tk1*) gene is a type of suicide gene that can be transferred to tumor cells. When exposed to systemically administered ganciclovir, the gene causes phosphorylation of the drug that results in death not only of transfected tumor cells but of surrounding tumor cells.[291,313,314] Through a limited institutional phase I study, Packer et al.[315] recently demonstrated the feasibility and safety of *HSV-Tk1* gene therapy in children with recurrent supratentorial malignant brain tumors. Significant toxicities associated with the injected vector or ganciclovir exposure occurred in 4 of 12 treated patients and included seizures, headache, lethargy, weakness, cerebral edema, and symptoms of increased ICP. All these symptoms resolved spontaneously or with a short course of glucocorticoid therapy.

Modulation of the immune response to brain tumors through the use of cells genetically modified for secretion of IL-2, interferon-g, tumor necrosis factor, and IL-4 has been relatively well studied preclinically and with mixed results.[312,316,317,318 and 319] IL-4 has emerged as a particularly potent antiglioma cytokine.[288] In an animal model, the efficacy of an IL-4-transduced 9L glioma cell vaccine in eradicating tumor and prolonging survival has been shown.[320,321] On the basis of these results, adult phase I trials will be initiated. Vaccine therapy using dendritic cells for antigen presentation also is under investigation.[322,323] Preliminary data indicate that such vaccines may be as potent as, or even more so than, those based on cells modified for cytokine production.[322]

Collectively, these preclinical and early clinical data support a continued investigation of the potential role for gene therapy against CNS tumors. The majority of work has been done with adult gliomas. Similar studies with high-risk pediatric brain tumors are eagerly awaited.

### Clinical Trials Groups

The use of chemotherapy for CNS tumors now is commonplace and, for specific tumors, is considered the standard of care (as discussed in later sections). However, for no single tumor can any *specific* regimen be considered standard. The COG, consisting of pediatric cancer programs from North America, Europe, and Australia, conducts numerous clinical trials of chemotherapy for nearly every brain tumor type and for children of all ages. New agents, or new schedules of established agents, are studied in clinical trials for the primary therapy of newly diagnosed disease or as treatment for recurrent disease. Correlative biologic studies are conducted on several tumor types. Similar national and international cooperative groups exist worldwide. In 1999, the National Cancer Institute established the PBTC from nine institutions that collectively diagnose disease in and treat the majority of U.S. children with primary brain tumors. The objectives of the PBTC are to develop pilot data rapidly about new therapeutic agents and neuroimaging techniques and from biologic studies of brain tumor specimens. Results from these studies will be available to the COG and other international cooperative groups for confirmatory testing in larger phase II and phase III studies.

Physicians and interested families can learn more about investigational chemotherapy protocols by contacting any of these cooperative groups or by contacting foundations that support pediatric brain tumor research. Details are presented at the end of this chapter under Information on Clinical Trials.

## TUMORS IN INFANTS AND YOUNG CHILDREN

Because of the special nature of the harboring of a CNS tumor in very young children and of the general practice of treating these children with protocols separate from their older counterparts with the same diagnoses, infants and young children are discussed as a separate group. In practice, the upper age of eligibility for infant studies varies generally between 18 months and several years.

### Demography

Approximately one-third of CNS tumors in children younger than age 15 years are diagnosed before the age of 5.[324] In the most recent SEER registry data reflecting incidence of brain tumors from 1975 to 1995, the annual incidence rates for brain tumors are highest in the first 5 years of life and are fairly constant from year to year.[1] As with other ages, infant boys are affected more commonly than are girls, but the ratio between them is dependent on both tumor type and age. For example, although medulloblastoma occurs overall more commonly in boys, girls are affected more commonly in the first year of life, with a ratio to boys of 1.27. Except for the fifth year of life, brain tumors are more common in whites than in blacks.[1]

### Special Clinical and Pathologic Considerations

Astrocytomas are the tumors that occur most commonly in infants, followed in incidence by PNETs (consisting primarily of medulloblastomas but including pineoblastomas and intracranial neuroblastomas as well), “other gliomas,” and ependymomas.[1] These registry data generally are reflected in the accrual onto the infant brain tumor trials open to all malignant diagnoses; in them, medulloblastoma and ependymoma are most common.[325,326,327 and 328]

Certain CNS tumors have a predilection for infants and young children. Among the low-grade gliomas, these tumors include optic chiasm and hypothalamic gliomas (discussed in the section Tumors of the Optic Pathway). The DIG, a mixed neuronal-glial tumor, frequently is mistaken for a more malignant process. Recognition of this entity is important because, despite its malignant appearance, complete resection alone appears to be curative and chemotherapy is not indicated.[115,150] The

DNET is another of the mixed tumors that occur more commonly in infants. Other less common low-grade tumors encountered primarily in infants and young children are the mature and immature intracranial teratomas.

Among the more malignant tumors, AT/RT primarily occur in children younger than 2 years. They occur more commonly in male children and in the cerebellum. Historically, treatment regimens have varied, but survival is poor. [108,329] Although the tumors are responsive to chemotherapy, the response is generally of short duration. Dissemination of disease along with local recurrence is the most common pattern of treatment failure, and survival generally is less than 1 year.

## Clinical Presentation and Differential Diagnosis

Although the presentation of CNS tumors has been described earlier, a few points concerning very young children bear repeating. The evolving anatomy of the infant skull, together with the predilection for supratentorial sites in the first 1 to 2 years, influences the presentation of these tumors. The principal calvarial sutures do not fuse until approximately 6 months of age. Until that time, tumors may be accommodated easily and not become clinically evident until they have reached a large size. An increasing head size, with or without a bulging fontanel, is particularly characteristic of tumors until the age of suture fusion. General signs of failure to thrive, such as poor feeding, emesis, and lethargy, are common and may precede large head growth. These symptoms may be mistaken for the much more common anatomic problems or infections of the gastrointestinal tract or infections of the ears or sinuses. Loss of developmental milestones, seizures, and hemiparesis can result from perinatal complications and cerebral palsy. A mass lesion can cause a hemiparesis manifested by early hand preference. The same manifestation may result from hypoxic brain injury. Evaluation of these general symptoms, particularly when no obvious cause is found or when they persist despite other intervention, should include imaging of the brain to rule out a CNS tumor.

## Treatment and Prognostic Considerations

### *Surgery*

The surgical management of brain tumors in infants follows the same general approaches as that indicated for the specific tumor types in older children. However, infant brain tumors pose a number of unique challenges that add to the difficulty and potential morbidity and mortality of surgical intervention. [330,331,332,333 and 334] First, these lesions often are fairly large at presentation, as described. Second, infant brain tumors are, as a group, more uniformly malignant than are those observed in older children and tend to be extremely invasive into the surrounding brain, increasing the chances of postoperative morbidity. Third, these lesions tend to be extremely vascular. This factor, coupled with the small blood volume of an infant, increases the risks from life-threatening hemorrhage during the course of the tumor resection. Fourth, because postoperative radiotherapy carries significant long-term sequelae in infants and young children, a greater onus is placed on surgeons to attempt as complete a resection as is safely feasible. The management of these tumors requires not only significant surgical expertise but skilled anesthetic management, with attention to adequate replacement of blood products and clotting factors during the resection.

### *Radiotherapy*

With the possible exception of those in LGAs, the survival rates from other CNS tumors in infants and young children have historically been lower than those in their older counterparts.[324,335] The reason for this finding may be biologically based for certain tumors but probably is due in equal measure to the administration of lower doses of radiotherapy because of concerns about long-term adverse sequelae. [336] Craniospinal irradiation to young children is associated with significant neuropsychological, neurodevelopmental, and neuroendocrine deficits that are more profound with earlier age at irradiation. [337,338,339,340,341 and 342] Recognition of these effects and their relation, in part, to radiotherapy led in the 1980s to investigational treatment approaches that relied on use of postsurgical chemotherapy to obviate the use of radiotherapy in primary therapy, to delay its administration, or to allow concurrent use at lower doses. This strategy resulted in cure rates higher than those in historical reports but, for infants with ependymoma and medulloblastoma, rates still were suboptimal. After progression of disease on chemotherapy, craniospinal irradiation provides control of disease in 25% to 30% of patients. [343] Because most instances of tumor progression or recurrence are at the local tumor site, current studies are examining the earlier use of conformal posterior fossa irradiation to improve local disease control. If chemotherapy can control microscopic disease successfully outside the primary tumor site, conformal radiotherapy offers the potential advantage of decreased neurotoxicity, as less normal brain will be irradiated.

### *Chemotherapy*

Published results of recent infant brain tumor trials using primary chemotherapy are listed in Table 27-7. Results of the first cooperative group [Pediatric Oncology Group (POG)] study to use postoperative primary chemotherapy, POG 8633, recently were updated. [344] For the patients as a whole, the most important prognostic factor was degree of tumor resection accomplished at diagnosis. The 5-year survival for all patients who had a GTR of their primary tumor, as determined by central review, was 62%; for those whose tumors were resected less than completely, the rate was 31%. Similar results were seen in the two most common diagnoses: For patients with medulloblastoma, the 5-year survival rates after GTR and less than GTR were 60% and 32%, respectively; for ependymoma, the corresponding figures were 66% and 25%, respectively. The presence of metastases did not independently predict outcome for either diagnosis. Except for ependymoma, younger age was not a prognostic factor. These and other smaller studies have demonstrated that, for children with medulloblastoma and ependymoma, the use of primary chemotherapy after complete resection of disease, with either delayed or lower-dose radiation, results in survival rates that approach those seen in older children.

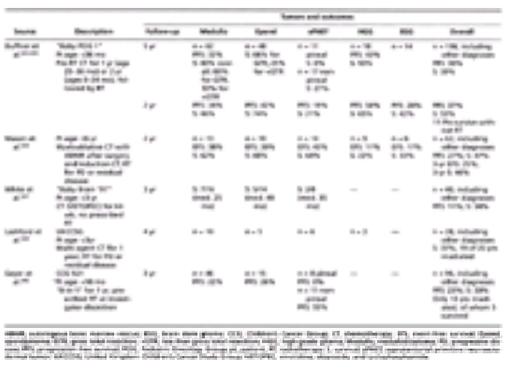


**TABLE 27-7. MULTI-INSTITUTIONAL STUDIES OF CHEMOTHERAPY FOR INFANTS AND VERY YOUNG CHILDREN WITH NEWLY DIAGNOSED BRAIN TUMORS**

Alternatively, chemotherapy has been used after reduced-dose craniospinal irradiation (CSI). In a small pilot study of ten patients, CSI was given with vincristine at 18 Gy to the craniospinal axis and 50.4 to 55.8 Gy to the posterior fossa; this was followed by 48 weeks of vincristine, cisplatin, and lomustine (CCNU) therapy. [345] Seven of the patients survived without recurrence at more than 6 years from diagnosis. The neurotoxicity with this approach was minimal; IQ scores 3 years after diagnosis were within 8 points of baseline scores.

In several noteworthy studies, a small minority of children, mostly with medulloblastoma, apparently were cured with surgery and chemotherapy alone. [325,327,346,347] The oldest of these series was initiated in 1976 and recently was updated by Ater et al. [347] Children who were younger than 3 years and had malignant brain tumors were treated for up to 2 years with mechlorethamine, vincristine, procarbazine, and prednisone; radiotherapy was reserved for recurrent disease. Of 12 patients with medulloblastoma, eight survived at a median of more than 10 years, and six of these never received radiotherapy. Of five patients with ependymoma, two survived, one without having received radiotherapy. Irradiated survivors had a mean IQ of 85, whereas those who were not irradiated maintained a mean IQ of 100.1. These studies, and that of Goldwein,[345] suggest that neurotoxicity can be reduced substantially in a subset of young patients with medulloblastoma by lowering, or even omitting, radiotherapy.

In both the POG and the Children's Cancer Group (CCG) infant brain tumor studies, histology of sPNETs affected outcome. Children with pineoblastoma in either study did not survive, whereas those with nonpineoblastoma, nonmedulloblastoma PNETs had prolonged survivals. [325,346] A similarly poor outcome has been seen with

other primary chemotherapeutic strategies for sPNETs.[348]

More recent U.S. and European clinical trials have incorporated dose-intensified chemotherapy after surgery and have either restricted or withheld radiotherapy. The benefit of this approach has not yet been proven. The second POG “Baby-Brain” study (POG 9233) randomly assigned patients prospectively to standard-dose chemotherapy, as was given in the first such study, or to a dose-intensive schedule of the same agents. Initial results indicate that the dose-intensive approach is feasible, is more toxic than the standard schedule, and does not appear to provide a survival benefit for children with medulloblastoma and ependymoma. [349] Event-free survival was significantly higher on the intensive schedule for infants with ependymoma. Again, complete resection of ependymoma and medulloblastoma was associated with significantly higher rates of event-free and overall survival. Mature data from that study, including those from the companion radiotherapy study, are eagerly awaited. (Dose intensification of chemotherapy with stem cell or marrow support was discussed in the section Drug Delivery Strategies.)

Currently, no chemotherapy approach to infants with malignant CNS tumors can be considered standard. The long-term results of POG 8633 are encouraging but have not yet been duplicated. The value of dose-intensified chemotherapy, as compared to standard doses, is not clear. New agents are sorely needed. The PBTC is studying the addition of intrathecal mafosfamide to a schedule of cisplatin, cyclophosphamide, vincristine, and oral etoposide, similar to earlier trials. Outside the clinical trial setting, the use of chemotherapy in standard doses for a limited period before radiotherapy is a reasonable approach but cannot be considered curative by itself. It appears that radiotherapy cannot yet be avoided. However, using the recent development of highly conformal delivery technology, current studies are testing hypotheses that earlier use of radiotherapy may lead to increased local tumor control rates and perhaps increased cure rates, while potentially decreasing long-term neuro-cognitive sequelae from decreased exposure of normal brain tissues to radiation.

Current infant trials also are seeking to determine whether the benefit of total tumor resection observed in other studies can be expanded to a larger proportion of patients. Limited data suggest that neoadjuvant chemotherapy can render tumors more easily resectable. [350] On the basis of these data and the results of earlier trials, ongoing studies are incorporating second-look operations into chemotherapeutic regimens.

New molecular technologies offer the hope that genetic profiles of tumors will help to guide clinical oncologists in their design of treatment protocols. If inherent tumor biology can be defined through genomic profiling, it might render possible identifying subsets of young children who might be curable with surgery and chemotherapy alone. Those with higher-risk disease warranting innovative treatment approaches also might be described. Ultimately, such molecular genetic analyses may permit more effective and less toxic scheduling of chemotherapy and radiotherapy.

## MEDULLOBLASTOMA

### Demography

Medulloblastoma accounts for approximately 20% of all primary pediatric CNS tumors and for approximately 40% of tumors arising from the cerebellum, and it is the most common malignant brain tumor of childhood. Although the tumor may be diagnosed in teenagers or young adults, most cases of medulloblastoma occur in the first decade of life. The peak age of incidence is between 3 and 4 years, and boys are affected between one and a half and two times as commonly as girls. [1]

### Pathology and Patterns of Spread

The ongoing debate surrounding the nomenclature of medulloblastoma and PNET was reviewed earlier. The most recent WHO classification of brain tumors maintains medulloblastoma as an independent cerebellar embryonal neuroepithelial tumor and classifies separately other similar-appearing embryonal small-cell tumors in other locations.[10]

Medulloblastomas are highly cellular, soft, friable tumors composed of cells with deeply basophilic nuclei of variable size and shape, little discernible cytoplasm and, often, abundant mitoses (Fig. 27-10). Homer Wright rosettes and pseudorosettes are variably present. Various degrees of glial or neuronal differentiation are noted, suggesting that the primitive cell of origin possesses the capacity for bipotential differentiation. [106,107] A histologic variant with an abundant stromal component, desmoplastic medulloblastoma (Fig. 27-11) occurs dominantly in the lateral cerebellar areas of adolescents and adults. [121,351]

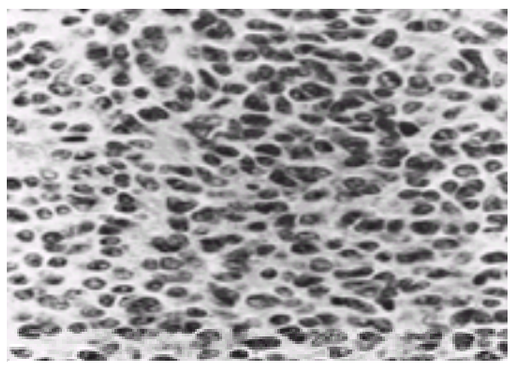


**FIGURE 27-10.** Typical histologic features of medulloblastoma (primitive neuroectodermal tumor). Tumor formed by apparently undifferentiated, basophilic, round to oval nuclei with minimal perceptible cytoplasm. (Hematoxylin and eosin, ×400.)

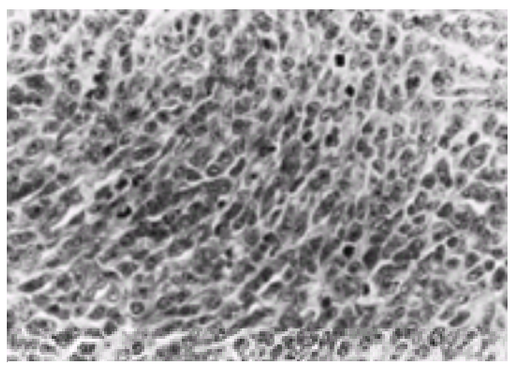


**FIGURE 27-11.** Desmoplastic medulloblastoma showing linear arrangement of cells along delicate background fibers. (Hematoxylin and eosin, ×400.)

An aggressive variant of medulloblastoma, termed *large-cell* and *large-cell anaplastic*, has been described.[352,353] As the names imply, the histologic features that distinguish this subset of medulloblastoma are large round nuclei with prominent nucleoli, frequent mitoses, abundant apoptosis and, in the anaplastic subset, nuclear pleomorphism. These tumors are uniformly positive for synaptophysin; positive staining with chromogranin is common as well. Monosomy 22 has not been seen in the cases described. The large-cell anaplastic variant represented 4% of the nearly 500 cases of medulloblastoma reviewed by Brown et al. [353]

Medulloblastomas often grow to several centimeters and may fill the posterior fossa, invading surrounding CNS structures as they occupy the regional subarachnoid and ventricular spaces. As with other CNS tumors of presumed primitive neuroepithelial origin, widespread seeding of the subarachnoid space may occur ( Fig. 27-12). The reported frequency of CNS spread outside the area of the primary tumor at diagnosis is 11% to 43%, and such spread eventually occurs in as many as 93% of patients who come to necropsy.[354,355]

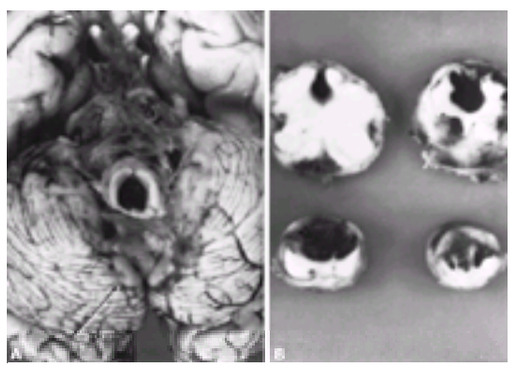


**FIGURE 27-12. A:** Nodules of a medulloblastoma (primitive neuroectodermal tumor) in the cisterna magna (*arrow*). A hemorrhage is present in the medulla. **B:** Transverse section of the medulla and spinal cord showing metastatic deposits of medulloblastoma in the subarachnoid space. Tumor is partially hemorrhagic and has invaded the neural tissue to a variable extent at the different levels.

Of all pediatric CNS neoplasms, the medulloblastoma has the greatest propensity for extraneural spread. Although this has been observed in 20% to 35% of patients in smaller institutional studies, more recent larger series suggest that the rate of such events is less than 4%. [195,356,357,358 and 359] Bone is the most common site, accounting for more than 80% of metastases; bone marrow, lymph nodes, liver, and lung are other common sites.

### Prognostic Considerations

An analysis of prognostic factors is confounded by the rapid diagnostic and treatment modality changes that have occurred over the last two decades. Assessment of biologic factors is only beginning. Some of the clinical and biologic characteristics of the disease that have, or have had, prognostic value in medulloblastoma are discussed here.

#### *Age at Diagnosis*

Determining the influence of age as an independent prognostic factor is difficult because of several observations: younger patients tend to have a higher rate of disease dissemination at diagnosis,[359,360] a lower overall rate of complete tumor resection,[325] and less aggressive radiotherapy.[336] In addition, more aggressive histologic subtypes of medulloblastoma[353] and AT/RT, which might be misdiagnosed as medulloblastoma, occur more commonly in the first few years of life. Nevertheless, children younger than 2 or 3 years have a worse prognosis than do older children. The age at which outcome no longer is affected negatively is not clear. In studies of patients older than 2 to 3 years, for whom radiotherapy generally is used as the primary postoperative treatment modality, younger age has been prognostic of outcome in some series[197,361] but not in others.[362,363]

#### *Tumor Size and Extent*

The Chang staging system, shown in Table 27-8, was developed in 1969 and used preoperative imaging studies, the surgeon's intraoperative impressions, and CSF cytology to assign stages of primary (T stage) and metastatic (M stage) disease. Modifications of the system have been proposed but, in any form, the Chang stage remains the system used most widely for designating the extent of disease.

| Stage | Definition |
|---|---|
| Tumor | |
| T1 | Tumor <3 cm in diameter and limited to the midline position in the vermis, the roof of the fourth ventricle and, less frequently, to the cerebellar hemispheres |
| T2 | Tumor >3 cm in diameter, further invading one adjacent structure or partially filling the fourth ventricle |
| T3 | Divided into T3a and T3b |
| T3a | Tumor invading two adjacent structures or completely filling the fourth ventricle with extension into the aqueduct of Sylvius, foramen of Magendie, or foramen of Luschka, thus producing marked internal hydrocephalus |
| T3b | Tumor arising from the floor of the fourth ventricle or brainstem and filling the fourth ventricle |
| T4 | Tumor further spreading through the aqueduct of Sylvius to involve the third ventricle or midbrain, or tumor extending to the upper cervical cord |
| Metastases | |
| M0 | No evidence of gross subarachnoid or hematogenous metastases |
| M1 | Microscopic tumor cells found in cerebrospinal fluid |
| M2 | Gross nodule seedings demonstrated in the cerebellar cerebral subarachnoid space or in the third or lateral ventricles |
| M3 | Gross nodular seedings in the spinal subarachnoid space |
| M4 | Extraneural metastasis |

**TABLE 27-8. CHANG STAGING SYSTEM FOR POSTERIOR FOSSA MEDULLOBLASTOMA**

Although M stage retains prognostic value (see later), T stage does not in most recent large series. [194,359,364,365] Brainstem involvement by direct infiltration of tumor, designated as Chang T3b, was historically indicative of a worse prognosis. However, with modern treatment regimens employing chemotherapy with radiotherapy, this feature is not prognostic. [193,196,364,366,367]

Extent of disease, or M stage, is consistently predictive of outcome, with two caveats. First, M1 disease, indicating positive CSF cytology without radiographic evidence of disseminated disease, is rare, and its impact on survival is unclear. In CCG study 921, wherein 18% of patients had M1 disease, 5-year progression-free survival (PFS) was 57% (± 10%), a rate lower than that for M0 disease and higher than that for M2 or M3 disease, but not significantly different in either case. [365] In the German HIT '91 trial, 13% of patients had M1 disease, and their survival rate of 65% (± 12%) did not differ from that of patients with M0 disease. [361] A similar rate of survival was found for the low number of M1 patients in the French M7 protocol for metastatic medulloblastoma. [363]

Of note is the observation that sampling of CSF from different sites may lead to discordant results. Positive cytology from ventricular CSF, obtained through ventriculoperitoneal shunts, does not correlate consistently with cytology from the lumbar space, which is more sensitive in detecting malignant cells. [368] Cytologic examination of CSF obtained by lumbar tap remains the standard method for determining CSF disease status.

Additionally, although M2 to M3 disease is prognostic in all series, ascertaining the independent impact of M4 disease, indicating extraneural metastases, is difficult. It is a much less common occurrence than is M1 disease and, in most recent medulloblastoma trials, patients with M4 disease were either excluded from, or not accrued to the study. As a result, M2 disease and M3 disease usually are combined in outcome analyses, often with M1 disease. In all series, a higher M stage of disease correlates with a lower survival rate. [359,361,362 and 363,365]

#### *Extent of Resection*

A near-total resection (generally defined as a more than 90%) can be achieved in approximately 80% of medulloblastomas using contemporary microsurgical techniques.[205] Evidence suggesting that extent of resection correlates with outcome has been provided by several single-institution studies and by a multi-institutional study of the International Society of Paediatric Oncology. [194,195 and 196,198,199,369] For example, Jenkin et al.[369] reported a 5-year PFS of 93% in children undergoing “gross total” resection versus only 45% in those undergoing incomplete resection. These observations were in contrast to older multi-institutional studies that failed to detect an effect of resection extent on outcome.[359,370] In neither of these older studies was M stage assessed uniformly, however. More recently, the CCG study 921 demonstrated a clear relationship between extent of resection and outcome but only in the subset of patients who had no evidence of tumor dissemination. [194] The same study found that, regardless of the surgeon's estimate of disease resection, the presence of less than 1.5 $cm^2$ residual disease on postoperative imaging was

associated significantly with a higher PFS rate in patients with M0 disease; this effect was greatest in children older than 3. [194]

Although the foregoing studies support the performance of extensive surgical resections in children with medulloblastoma, little convincing evidence substantiates improved outcome when gross total is compared with near-total resections. This distinction is important, because these tumors generally infiltrate the floor of the fourth ventricle. The observation that this microscopic residual disease does not affect prognosis adversely provides a strong rationale for avoiding aggressive removal of small components of brainstem disease, thereby minimizing the morbidity of the resection.

### *Shunts*

Although some reports have suggested that shunts placed to relieve hydrocephalus are associated with an increased incidence of systemic metastases, [195,356,371] other studies have cast doubt on this association. [354,372] A more recent review demonstrated fairly conclusively that, in patients with comparable risk factors, insertion of a shunt did not appear to increase the risk of systemic tumor dissemination. [180] Ventriculoperitoneal shunts and alternatives to relieving hydrocephalus have already been discussed.

### *Molecular Markers*

Several recent investigations have identified molecular markers that may be more accurate than clinical criteria for predicting medulloblastoma prognosis. Early flow cytometry studies indicated an association between aneuploidy and a more favorable prognosis. [373] Later cytogenetic studies further refined the link between genomic mutations and tumor behavior, demonstrating that deletions of chromosome 17p were associated with poor outcome. [90] Amplification of c-*Myc* was linked strongly to poor prognosis but was found to occur in fewer than 20% of all medulloblastomas. [374]

Several recent investigations have focused on gene expression as a marker of medulloblastoma prognosis. Expression of the neurotrophin-3 receptor TrkC has been found to be associated with a favorable prognosis. [375,376] and [377] The majority of medulloblastomas expresses both TrkC and neurotrophin-3, suggesting an autocrine or paracrine receptor activation of the TrkC receptor tyrosine kinase. [375,377] Moreover, TrkC activation induces medulloblastoma apoptosis that possibly may contribute to the more favorable prognosis of tumors with high TrkC expression. [377] In contrast, a poorer prognosis has been linked to increased expression of the neuregulin receptors *Erb*B-2 and *Erb*B-4 and of c-*myc*.

These discoveries suggest that inherent biologic differences, reflected in the divergent expression of genes that regulate tumor growth and response to therapy, determine the clinical outcome of morphologically similar-appearing medulloblastomas. Efforts now are under way, through multi-institutional therapeutic trials, to test prospectively molecular markers that might be used for the stratification of patients in future clinical investigations. Furthermore, the molecules identified by these investigations might serve as targets for future, biologically based therapies specifically designed on the basis of molecular mechanisms regulating tumor growth.

### *Risk Groups*

Most clinical trials in the United States stratify patients on the basis of the following factors for treatment strategies: age less than 3 years, M+ disease, and more than 1.5 $cm^2$ disease residual on postoperative images (generally indicating higher-risk disease); and age no younger than 3 years, M0 disease, and less than 1.5 $cm^2$ of disease residual (indicating lower-risk disease). All these factors are clinical and, thus far, biologic characteristics of disease, including histologic variants of medulloblastoma, have not figured into risk classification.

## Treatment

### *Surgery*

A fundamental decision in evaluating a child with a posterior fossa tumor is determining whether the tumor is a mass lesion arising from the cerebellar hemisphere, vermis, or fourth ventricular floor or is an intrinsic tumor of the brainstem. Children with the former lesion types (e.g., medulloblastomas, ependymomas, cerebellar astrocytomas) undergo surgical intervention to allow for diagnosis and to achieve cytoreduction.

In children with a resectable lesion, such as a medulloblastoma, the timing of surgery is determined by the clinical status of the children. If they are alert, high-dose corticosteroids are administered and, if feasible, a spine MRI scan is obtained to determine the presence of evidence of leptomeningeal dissemination; the craniotomy then is performed on the following day. This approach is reasonable also if such children are lethargic but become alert after administration of corticosteroids. However, in the rare situations in which such children are extremely somnolent, urgent surgical intervention is preferred.

A ventriculostomy is placed in most cases for temporary CSF diversion, and this is “weaned” by gradual elevation of the drip chamber during the postoperative period. If affected children need CSF drainage for more than 7 days after tumor removal, permanent CSF diversion usually is performed.

The tumor usually is approached through a suboccipital craniotomy or craniectomy, with the patient in a prone or modified prone (Concorde) position. The dura is opened in a Y-shaped fashion. Cerebellar hemispheric lesions, which are encountered most commonly in older children, are exposed fully by a transverse or vertical incision in the cerebellar hemisphere. The more common vermian and fourth ventricular lesions may be visible within the foramen of Magendie but, if not, are exposed by dividing the caudal 1 to 2 cm of the inferior vermis. Because approximately 10% of patients develop a postoperative syndrome of pseudobulbar symptoms and mutism (the “posterior fossa syndrome”) after vermian tumor resections [378,379] —a reaction possibly related in part to the extent of the vermis incision—an attempt is made to incise only as much vermis as is needed to provide adequate exposure of the tumor. The central portion of the lesion then is debulked using the ultrasonic aspirator. The plane between the tumor and the vermis then is followed rostrally until the roof of the fourth ventricle is reached. Once the tumor has been debulked partially, a cottonoid patty is inserted under the caudal aspect of the tumor along the floor of the fourth ventricle, to minimize the risk of injury to the brainstem as additional tumor is removed from within the fourth ventricle. Some surgeons monitor electromyelograms of the lateral rectus and facial muscles during the resection to reduce the risk of abducens and facial nerve palsies. Next, tumor extending laterally within the foramina of Luschka or the cerebellar peduncles is removed. Finally, tumor adherent to the floor of the fourth ventricle is removed carefully. The aggressiveness of the resection in this region is guided by the frozen-section diagnosis. For medulloblastomas, the tumor can be shaved down to the floor of the fourth ventricle, but removal of tumor below the floor is unnecessary as it does not appear to improve outcome and clearly increases the potential for morbidity. This recommendation contrasts to the same situation for ependymoma (discussed later).

Once the tumor has been removed, the dura is closed, generally with a graft. Because a CSF examination is an important component of the postoperative staging of children with medulloblastomas and because blood and debris can settle in the lumbar thecal sac within several hours of surgery, complicating interpretation of the cytology for several weeks, many groups either perform a lumbar puncture immediately after the tumor resection or delay the puncture until the third postoperative week. Currently available data are insufficient to judge whether intraoperative CSF sampling from the cisterna magna is adequate for staging of disease.

### *Radiotherapy*

Medulloblastoma is radiosensitive; hence, for decades, the standard curative therapy for medulloblastoma has been craniospinal irradiation. Attempts at not irradiating the entire neuraxis or omitting radiotherapy altogether have resulted in compromised survival. [380] Conversely, craniospinal irradiation can produce intellectual and endocrinologic morbidity, most marked in younger patients. Therefore, the desirable approach is to use a minimum craniospinal radiation dose that does not jeopardize the cure rate. A small, prospective, multicenter study in which patients with favorable prognostic factors were randomly assigned to the standard (36.0-Gy) irradiation versus reduced-dose (23.4-Gy) neuraxial irradiation showed an excess number of neuraxial relapses in the reduced-dose arm. However, the difference in survival rates between the two arms was only of borderline statistical significance. [381] Whether the addition of chemotherapy to reduced-dose craniospinal irradiation would result in outcomes similar to standard-dose irradiation is unknown. One randomized study to test this hypothesis was aborted because of insufficient patient accrual secondary to physician and family bias in favor of chemotherapy. Nevertheless, the current COG protocol uses 23.4 Gy and randomly assigns patients to one of two different chemotherapeutic regimens. Hyperfractionated neuraxial irradiation has not been proven to be superior. Undue protraction of the course of radiotherapy has been shown to have an adverse impact on the control of the disease. [382]

The radiation dose to the posterior fossa tumor bed is less controversial. A total dose of less than 50 Gy has been shown to lead to inferior cure rates. The dose used most commonly is 54.0 to 55.5 Gy. The potential benefit of an additional radiosurgical boost in high-risk patients is still under investigation. A current debate concerns

whether the entire posterior fossa or just the tumor bed plus a margin must receive the full dose of radiation. The bulk of medulloblastoma recurrence is in the posterior fossa or adjacent to the original tumor site. Therefore, a reasonable approach is to deliver the full dose to the tumor bed plus a 2-cm margin only. Modern conformal techniques, such as IMRT, can deliver the full dose to such a target while reducing the radiation dose to the cochleas and hypothalamic-pituitary axis. A preliminary study using IMRT has shown a significant reduction of ototoxicity despite the addition of cisplatin chemotherapy (S. Woo, unpublished data).

The technique of craniospinal irradiation is well-known. However, ensuring at least a 5-mm margin of coverage of the cribriform plate is important, as inadequate irradiation of that area can lead to an increase in recurrence at this location. [383] Also recommended is MRI of the spine to determine the inferior border of the thecal sac in each patient.

### *Chemotherapy*

The potential benefit of chemotherapy to subsets of patients with medulloblastoma was demonstrated first by the International Society of Paediatric Oncology and was confirmed further in studies by the CCG and the POG.[196,359,370,384] Since those reports, imaging and surgical technology have advanced significantly, and tumor assessment has become more standard. Through a series of subsequent trials, medulloblastoma has emerged as one of the most chemotherapy-sensitive of all brain tumors. The tumor's sensitivity has allowed the investigation of chemotherapy use in different contexts: to increase disease control and patient survival; to decrease adverse effects of radiotherapy; and to postpone or obviate the need for radiotherapy in the very youngest child. Table 27-9 outlines more recent studies that have employed chemotherapy for the treatment of medulloblastoma. The agents used most commonly are the classic alkylators and platinum complexes. For these, issues of timing, use with other agents, and drug schedules are not yet standardized. However, toxicities limit the extent to which these agents can be used, and cure rates remain suboptimal for many patients. New agents that are more effective and less toxic are needed.

**TABLE 27-9. RESULTS OF RECENT MULTI-INSTITUTIONAL CLINICAL TRIALS OF CHEMOTHERAPY FOR NEWLY DIAGNOSED MEDULLOBLASTOMA**

With regard to increasing disease control, chemotherapy has been employed in both neoadjuvant and adjuvant settings. In patients with residual or metastatic disease, studies of neoadjuvant chemotherapy offer the potential advantages of identifying active agents; of delivering chemotherapy to a BBB that is possibly more advantageously disrupted owing to surgery; of treating micrometastatic disease throughout the CNS; and of decreasing gross residual tumor burden before the delivery of conventional radiotherapy. Chemotherapy before irradiation is better tolerated by the nonirradiated bone marrow and hearing apparatus as well. Several active drugs and drug combinations have been identified through neoadjuvant chemotherapy trials: cyclophosphamide [385]; cisplatin and vincristine [386]; "8-in-1"[387]; cisplatin and etoposide [388]; cisplatin, vincristine, and cyclophosphamide [389]; and carboplatin with etoposide.[390] The potential benefit of decreasing tumor burden before craniospinal radiotherapy is accompanied by a risk of disease progression before initiation of radiotherapy. Progressive disease rates of 20% to 30% have occurred during various neoadjuvant regimens.[365,389,390 and 391] Whether progressive disease or disease improvement during neoadjuvant chemotherapy ultimately affects long-term disease control is not known. However, delay of radiotherapy may be detrimental to the outcome of certain patients, as suggested in a study by Kortmann.[361]

Medulloblastoma's sensitivity to chemotherapy has rendered possible the modulation of treatment modalities based on relative risk of recurrence in balance with potential long-term sequelae of treatment. In this context, chemotherapeutic trials generally have employed two risk strata for purposes of treatment: higher-risk disease, defined as that which is resected incompletely or is metastatic, and lower-risk disease, defined as completely resected and without metastases. The goal of adding chemotherapy to low-risk disease is to allow a safe reduction of radiation dose outside the posterior fossa so as to decrease the risk of late, adverse neuro-cognitive effects due to radiation. Available data from the POG/CCG study of standard (36-Gy) radiotherapy versus reduced-dose (23.4-Gy) radiotherapy, without the addition of chemotherapy, for lower-risk medulloblastoma patients indicate that neurotoxicity is indeed lower after reduced-dose radiotherapy. [339] Whether the addition of chemotherapy to irradiation increases the survival of lower-risk patients is not clear. Such a benefit was suggested in Packer's limited institutional study,[362] which used chemotherapy in addition to standard-dose radiotherapy for patients considered at higher risk for disease recurrence. Many patients in that study, including those with M0 tumors and with totally or near-totally resected tumors, subsequently were considered to have lower-risk disease. The apparent benefit of chemotherapy to these patients has not been confirmed in larger controlled clinical trials.

In 1993, the CCG and the POG tried to answer the question through a randomized study of standard radiotherapy versus reduced-dose radiotherapy plus chemotherapy for low-risk patients. The study was stopped early, owing to lack of patient accrual secondary to parental and physician bias in favor of chemotherapy.[392] The current COG trial compares two cisplatin-based adjuvant regimens, substituting cyclophosphamide for CCNU in one arm, after reduced-dose craniospinal irradiation. Future trials will examine further reduction of radiotherapy dose. Outside a clinical trial, the use of chemotherapy for lower-risk patients could be considered a standard of care. However, the lowest, safest dose of radiotherapy to use within this approach has not yet been determined.

Chemotherapy studies for patients with higher-risk disease have focused on increasing tumor control and patient survival. In some series, the addition of chemotherapy to craniospinal irradiation has increased survival rates over those achieved with standard irradiation alone. [196,359,393] Conversely, reduction of craniospinal radiotherapy dose, in conjunction with adjuvant multi-agent chemotherapy, may result in lower survival rates. [394] Ongoing studies for high-risk patients are exploring chemotherapy dose intensification. The feasibility of administering several courses of high-dose chemotherapy with peripheral blood stem cell support after irradiation recently was demonstrated.[395] A similar approach is being studied in the COG as well. The effects of new agents on measurable disease and the addition of intrathecal chemotherapy also are being explored.

## SUPRATENTORIAL PRIMITIVE NEUROECTODERMAL TUMORS

The term *primitive neuroectodermal tumor* was coined by Earle and Hart in 1973. The histologies of all these tumors are similar, but their nosology has been controversial, as noted previously. Various designations of these tumors have been based on the site of tumor origin and by lines of differentiation within the tumor. Thus, the sPNET also has been called *cerebral* or *central neuroblastoma*, *cerebral medulloblastoma*, and *pineoblastoma*. Ependymoblastoma commonly has been included in the sPNET category as well. Medulloblastoma is the designation for PNET of the posterior fossa. In this section, only sPNET is reviewed.

H4>Demography

Collectively, sPNETs in childhood are rare, accounting for only 2.5% to 6.6% of CNS tumors in most series. [338,396,397,398 and 399] These tumors are more common during the early first decade.[330] The median age of onset varies between several months and several years in combined series of patients. [397,400,401 and 402] Many series show a male-female ratio near unity; in others, male persons predominate nearly 2:1. [397,402] Whites appear to be affected much more commonly than are nonwhites.[403]

### Pathology and Patterns of Spread

Despite the presence of considerable microscopic extension, sPNETs generally appear as well-circumscribed masses. Grossly, they are lobulated, soft, hemorrhagic, and often cystic masses. Microscopically, sheets of uniform embryonal-appearing, small, round cells with hyperchromatic oval nuclei and frequent mitoses are noted

(Fig. 27-13). Homer Wright rosettes and perivascular pseudorosettes and areas of necrosis are common. Although foci with various degrees of glial, neuronal, or ependymal differentiation are seen in 70% of tumors, light microscopy and ultrastructural evaluation generally reveal most individual cells to be primitive and undifferentiated.

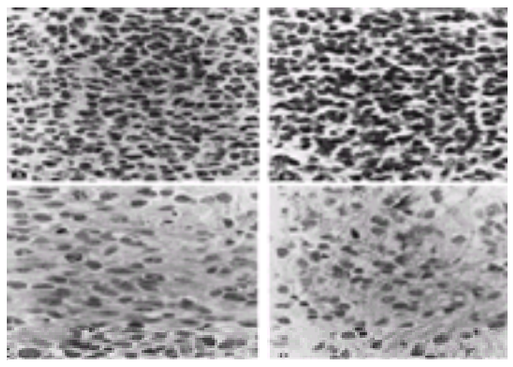


**FIGURE 27-13. A:** Typical field of medulloblastoma (primitive neuroectodermal tumor of the posterior fossa). Tumor is formed by apparently undifferentiated, basophilic, round to oval nuclei with minimal perceptible cytoplasm. (Hematoxylin and eosin, ×400.) **B:** Typical field of pineoblastoma (primitive neuroectodermal tumor of the pineal). Note the similarity to the photomicrograph in **A**. (Hematoxylin and eosin, ×400.) **C:** One of several nests of malignant astrocytes in primitive neuroectodermal tumor of the cerebrum, displaying mild pleomorphism and several mitotic figures. (Hematoxylin and eosin, ×400.) **D:** Another similar field from the same tumor shown in **C** stained with glial fibrillary acidic protein (GFAP) showing a few cells staining for GFAP. (GFAP, ×400.)

Wide local and regional tumor extension is common, and transcallosal extension into the opposite hemisphere also has been reported. [404] Although the incidence of diffuse leptomeningeal or spinal subarachnoid disease was as high as 30% at diagnosis in many early studies, [400,405,406,407,408,409,410,411,412] and [413] reliable statistics for the incidence of metastatic disease are not available, owing to the relative rarity of these tumors and to changes in diagnostic imaging modalities and practice over the last few decades.[414] Despite the initially low incidence of dissemination at diagnosis, the eventual occurrence of leptomeningeal spread may be as high as 70% at relapse. Systemic metastases have not been reported commonly, but they appear to favor bone and lung.

### Prognostic Considerations

Much of what is inferred about sPNETs is derived from the experience with medulloblastoma. In past and current protocols, treatment of these tumors has been similar. Whether such an approach is biologically sound is not known, because treatment outcomes for sPNET appear to be worse than those for medulloblastoma. From various series, different factors affecting outcome emerge. In the CCG 921 study of radiotherapy and two different chemotherapeutic regimens, site of primary tumor and the absence of metastases predicted a higher chance of survival.[403] Children with pineal tumors had a 3-year PFS rate of 61%, whereas for those with tumors of nonpineal origin, the rate was only 33%. Noteworthy is that whereas pineal site is favorable in older children, pineal location in infants portends a dismal prognosis (as noted earlier).[325,346] In the CCG study, PFS for nonmetastatic tumors, when adjusted for site, was 50%; for those with M+ disease, PFS was 0%.[403] GTR of disease, or no residual disease measured by postoperative MRI, is associated significantly with a better outcome in some series, [193,401] but other studies have failed to identify such an association.[346,404,411,412] Given the small numbers of patients with primary pineal sPNET and the inherent risks associated with operation in this area, assessing the benefit of tumor resection conclusively is difficult. [414]

### Treatment

#### *Surgery*

Because sPNETs often are large, highly invasive of functionally important cortex, and fairly vascular, gross total and near-total tumor resection are less frequent for sPNET than for medulloblastoma.[193] Furthermore, as stated, data are mixed with regard to the benefit of GTR of these tumors. Accordingly, the surgical management for sPNETs is similar to that for other cerebral malignant tumors, such as high-grade glioma, in which the primary goal is to relieve local mass effect by removing as much tumor as is safely feasible.

#### *Radiotherapy*

Standard therapy for patients with sPNET is craniospinal irradiation. Current treatment recommendations are craniospinal irradiation to 36 Gy with a boost to 54 Gy to the area of the initial tumor. Radiotherapy alone, as the only postoperative treatment, has been reported in only two retrospective studies; only 1 of 22 patients in the combined studies survived beyond 2 years.[415,416] Most treatment series have used both irradiation and adjuvant chemotherapy. Despite the improvements in survival rates noted with combined-modality therapy, PFS is no better than 33%. [193,396,403,404]

Craniospinal doses of 24 Gy and tumor doses of 45 Gy have been used in children younger than 3 years. As with medulloblastoma, it is uncertain whether the poorer outcome for this group of patients is related to the reduced radiotherapy dose or to more aggressive tumors that appear to have a higher incidence of dissemination at diagnosis.

#### *Chemotherapy*

Various single-agent and combination chemotherapeutic regimens have shown activity against sPNET. [346,388,407,417,418] No study has been designed to compare outcomes of medulloblastoma and sPNET statistically but, in recent series, the outcome for patients with sPNET appears to be inferior. In the CCG 921 study in which neoadjuvant and adjuvant “8-in-1” chemotherapy was compared to adjuvant CCNU, prednisone, and vincristine, each given with radiotherapy, 3-year PFS and survival were 45% and 57%, respectively, for all patients with sPNET and did not differ significantly between the two study arms. [403] Unlike the experience with medulloblastoma, neither degree of surgical resection nor extent of residual disease was prognostic. The authors concluded that the data were insufficient to determine whether the use of chemotherapy increased survival from that historically reported with surgery and radiotherapy alone. [404] Ongoing clinical trials offer combined-modality therapy to infants and older children with sPNET. Identification of either clinical or biologic prognostic factors is needed to redirect therapy in the future.

## EPENDYMOMA

### Demography

According to recent SEER registry data, ependymomas constitute approximately 9% of all primary CNS tumors in children. [1] The tumors usually arise within or adjacent to the ependymal lining of the ventricular system or the central canal of the spinal cord. Ninety percent of the tumors are intracranial, and up to two-thirds of these occur in the posterior fossa.[186,420,421] In children, the highest incidence occurs in the first 7 years of life [1]; the incidence peaks again in the third to fifth decades of life.[422] Whereas in the past, the ratio of male to female patients was reported to be near unity, recent series report a male-female ratio of between 1.3 and 2.0.[1,184,188,423,424] Of note in the potential etiology of these tumors is that DNA sequences identical to the SV40 polyomavirus and the corresponding viral large T-antigen have been found in several tumors; SV40 and related polyomaviruses can induce ependymoma and choroid plexus tumors in monkeys and other mammalian species.[425]

### Pathology and Patterns of Spread

Ependymomas generally are well-demarcated tumors that often display areas of calcification, hemorrhage, and cysts. Although uncommon, the ependymal rosette is a characteristic and diagnostic microscopic feature composed of radially aligned ependymal elements about a central lumen. More common is the conspicuous pseudorosette (Fig. 27-14), an eosinophilic halo composed of cells with tapering processes surrounding a blood vessel.

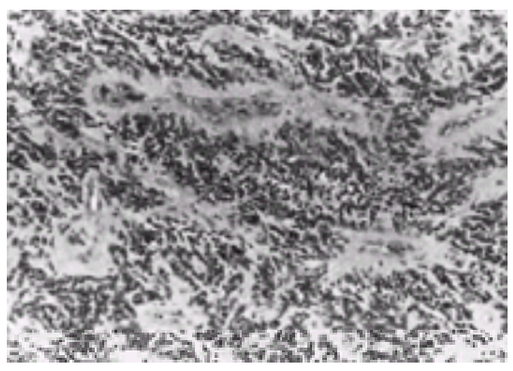


**FIGURE 27-14.** Section from a fourth ventricular ependymoma displaying typical perivascular pseudorosettes. (Hematoxylin and eosin, ×400.)

Ependymomas vary from well-differentiated tumors with no anaplasia and little polymorphism to highly cellular lesions with significant mitotic activity, anaplasia, and necrosis that may resemble glioblastoma multiforme. Ependymomas have been classified descriptively as cellular, epithelial, or papillary. Neither these terms nor a proposed classification that grades these tumors on the basis of their degree of anaplasia has gained wide acceptance, although they often appear in older literature. An exception to the use of such terminology exists for the ependymal tumors arising in the region of the conus medullaris and filum terminale, termed *myxopapillary tumors* because of their unique histologic features. Current terminology for ependymomas located elsewhere distinguishes between benign (low-grade) tumors and malignant (high-grade or anaplastic) tumors. The WHO classification system uses the terms *ependymoma* and *anaplastic ependymoma* to distinguish these two types. Although this division has been thought to correlate well with clinical outcome, its prognostic significance may be an artifact of the inclusion of a different but similar-appearing entity—the ependymoblastoma—within the anaplastic group. When this tumor has been removed from the series of ependymoma, the relationship between histology and clinical outcome generally disappears. [351,424,426,427]

Ependymomas are locally invasive tumors that spread contiguously into adjacent brain. Tumors arising in the posterior fossa frequently infiltrate the brainstem. In as many as one-third of these cases, tumor may project through the foramina to involve the medulla and upper spinal cord. [424] The incidence of spinal subarachnoid dissemination has been estimated to be 7% to 12% in combined patient series. One meta-analysis suggests that such events are potentially most common in high-grade and posterior fossa tumors.[422,428] Systemic metastases are rare and, when present, show a predilection for liver, lung, and bone.

**Prognostic Considerations**

The single most important prognostic factor that emerges from review of single- and multi-institutional experience with ependymoma is the extent of tumor resection.[182,183,185,186,187 and 188,344,420,428,429,430 and 431] Whether gauged by the surgeon's estimate or measured by postoperative MRI, patients whose tumors are grossly totally resected and those who have less than 1.5 $cm^2$ of residual disease on imaging have a rate of survival higher than that in children whose tumors are resected less than completely. The rates of survival after complete and less-than-complete resections range from 66% to 75% and 0% to 11%, respectively. [182,183,184,185,186,187 and 188] Perhaps related to degree of resection has been the finding in some series that location of primary intracranial tumor and patient age are prognostic. [432,433] However, these data are inconsistent except that the best prognosis is associated with ependymomas of the spinal cord. Younger children are more likely to have tumors arising from the posterior fossa and, in this location, tumors tend to be more invasive, and removing them entirely is more difficult. When lower age (usually younger than 2 to 4 years) has been found to have a negative impact on survival, this finding also has been attributed to the delivery of lower radiation doses. Another inconsistent prognostic factor is histology of the tumor; in some series, anaplastic or malignant histology predicts a poorer prognosis than does well-differentiated ependymoma,[182,420,428] although other series show no difference in outcome between the two histologic types. [183,186,187,424,426,434,435 and 436]

**Treatment**

***Surgery***

Techniques for the resection of posterior fossa ependymomas are similar to those used for resecting medulloblastoma, although the rationale for intraoperative monitoring of evoked potentials and cranial electromyography may be even greater because of the higher frequency of brainstem infiltration. Supratentorial ependymomas, often located subcortically, are resected in a fashion similar to that used in other deep-seated gliomas (described later).

The prognostic benefit of complete tumor resection has been stated. However, such a result has been feasible in only approximately 50% of ependymomas in most series. GTRs generally are more difficult for posterior fossa ependymomas than for supratentorial ependymomas because of the propensity for infratentorial lesions to infiltrate the brainstem and to surround cranial nerves and vessels lateral and ventral to the brainstem, precluding complete removal without unacceptable neurologic morbidity. Infants are particularly likely to have large infratentorial ependymomas with significant ventrolateral extension, which in part accounts for their less favorable prognosis in most series. [421,437,438,439 and 440] In such patients, multiple lower cranial nerve palsies as a result of both tumor and surgery often necessitate a tracheostomy and gastric feeding device. Resolution (if any) of neurologic impairment may be delayed for several weeks to months. [438,441] Because the prognosis in children with incompletely resected ependymomas is so poor, several recent treatment protocols have selectively incorporated second-look surgery in children with objective evidence of residual disease after an initial procedure. This generally has been attempted after a short course of neoadjuvant chemotherapy, administered in the hope of reducing the vascularity and invasiveness of the residual disease. A multi-institutional study, designed to examine the ability of this approach to improve outcome in such children without an unacceptable trade-off of morbidity, is currently being developed in the COG.

***Radiotherapy***

Local postoperative radiotherapy increases the overall survival rates of patients with ependymoma from between 15% and 25% to between 35% and 63%. Patients with supratentorial lesions should receive wide local treatment to 50 to 55 Gy. Because the tumor may spread by the subependymal route, the target volume for at least part of the treatment should include the wall of the lateral ventricles if the tumor involves any part of the ventricular wall. Posterior fossa tumor often extends down the cervical spinal cord, and careful attention should be paid to cover the involved upper cervical spine in the treatment fields.

Although a debate still questions the need for routine craniospinal irradiation, most investigators agree that patients without evidence of subarachnoid disease at diagnosis do not need craniospinal irradiation. Recent retrospective studies did not demonstrate a significant impact of prophylactic craniospinal irradiation on the rate of subsequent spinal dissemination or survival, regardless of tumor differentiation or primary site. [182,424,442] In intramedullary spinal cord or cauda equina ependymomas, postoperative radiotherapy could be withheld if complete surgical excision is accomplished.

***Chemotherapy***

Single- and multi-agent chemotherapeutic regimens have been used in ependymoma therapy. In clinical trials, the platinum agents appear to have the greatest activity. (For a comprehensive review of chemotherapeutic experience with ependymoma, the reader is referred to a review by Bouffet and Foreman. [443] The use of chemotherapy for infants with ependymoma is discussed earlier.) Despite the demonstrated activity of various regimens, the use of chemotherapy has not improved the survival of patients with either completely or incompletely resected ependymoma in any multi-institution series. For older children, chemotherapy is recommended only as part of a clinical trial.

## LOW-GRADE SUPRATENTORIAL AND CEREBELLAR GLIOMAS

Low-grade glial neoplasms are a diverse group of tumors that include JPA, fibrillary (also called *protoplasmic* or *diffuse*) astrocytoma, oligodendroglioma, ganglioglioma, and such mixed tumors as oligoastrocytoma. Their unifying features are their generally slowly evolving, nonaggressive clinical behavior and relatively benign histologic appearance. Generally high rates of long-term survival are characteristic as well, despite low but steady rates of disease progression even after 10 years from diagnosis.

### Demography

Cerebellar astrocytomas are the most prevalent, representing 15% to 25% of all CNS tumors, followed in prevalence by cerebral hemispheric astrocytomas and tumors of deep midline structures (each representing 10% to 15% of all CNS tumors) and tumors of the optic pathway (accounting for approximately 5% of all CNS tumors).[444] Seventy to seventy-five percent of cerebellar astrocytomas occur in childhood, [445,446] most commonly in the first decade of life. The average age at diagnosis ranges from 6.5 to 9.0 years. [143,446,447,448,449 and 450] Boys are affected more commonly than are girls.[451] Neuraxis dissemination of low-grade gliomas from any location in the brain is distinctly uncommon, occurring in only approximately 5% of cases. [452,453,454,455 and 456] Tumors arising from the hypothalamus and periventricular areas may be more likely to disseminate.

### Pathology and Patterns of Spread

Classifications, such as that of Kernohan, St. Anne/Mayo, and WHO, identify low-grade tumors primarily on the basis of their grade or degree of anaplasia rather than on histologic type.[95] Neoplasms that are only modestly cellular and contain few or none of the histologic criteria of malignancy are designated as low-grade or grade I and grade II lesions in these classifications. The WHO classification uses the grade I designation for the typical pilocytic astrocytoma and grade I or grade II for the mixed neuronal-glial tumors. The low-grade fibrillary astrocytomas that make up most adult low-grade lesions are designated as grade II. Grade III and grade IV tumors are high-grade lesions characterized by aggressive clinical behavior and malignant histology (considered separately in the section, Supratentorial High-Grade Gliomas). Although the utility of such grading systems has been questioned because of their subjective nature and their reliance on often small biopsies from tumors that may be heterogeneous, these systems remain popular because applying and understanding them is simple and because they have some prognostic value.

#### *Supratentorial Low-Grade Gliomas*

Most supratentorial low-grade gliomas are astrocytomas, a diverse group of neoplasms generally composed of GFAP-positive bipolar or stellate cells. Such designations as *fibrillary*, *protoplasmic*, *gemistocytic*, *xanthomatous*, and *pilocytic* often are used to describe the appearance of the astrocytes and their various histologic patterns.[95,457] Only the pilocytic and fibrillary varieties are seen commonly in children. Anaplastic transformation of these tumors leading to more malignant-appearing and clinically aggressive entities, such as anaplastic astrocytoma and glioblastoma multiforme, a common event in adults, occurs less frequently in children and young adults.[351,458,459 and 460]

Oligodendrogliomas, a separate category of glial neoplasms, are characterized by a generally monotonous collection of uniform spheroidal cells with more homogenous nuclei than are seen in the fibrillary astrocytoma, the tumor that represents the principal diagnostic alternative. An abundant clear cytoplasm surrounding a dark nucleus produces the appearance of a perinuclear halo that gives a distinctive fried-egg appearance. As in astrocytomas, grading of oligodendrogliomas appears to identify groups with differing prognoses. Although several schemes for grading have been proposed, no consensus over which tumor features are the most prognostically significant has emerged. Most investigators reserve the use of the terms *high-grade* and *anaplastic* oligodendroglioma for tumors with increased cellularity, mitotic activity, and other features of more malignant glial tumors (outlined earlier and in the section dealing with malignant gliomas). [461] In children, at least one-half of oligodendroglial tumors occur as a part of mixed astrocytic-oligodendroglial tumors. These tumors have a predilection for the frontal and temporal lobes. [462]

Mixed neuronal-glial cell neoplasms, such as ganglioglioma, gangliocytoma, DNET, and DIG, are grouped for convenience with the low-grade gliomas (discussed in the section, Supratentorial Low-Grade Gliomas). Gangliogliomas are the predominant type of mixed tumors, and they are composed of glial tissue and a disorderly array of binucleate ganglion cells that must be distinguished from normal neurons in the area involved. The glial component most commonly is astrocytic, but it may be oligodendroglial. Although anaplastic gangliogliomas are uncommon, when they do occur, they usually involve anaplasia within the glial component; anaplastic involvement of the neuronal component is unusual.[463]

#### *Low-Grade Cerebellar Tumors*

Low-grade gliomas occurring in the cerebellum typically are astrocytomas. Two principal histologic variants have been described. The classic, or pilocytic, astrocytoma accounts for 80% to 85% of these tumors (Fig. 27-15), and it is composed of fusiform astrocytes loosely interwoven with a fine fibrillary background and no (or rare) mitoses. A frequent microcystic component and the presence of Rosenthal fibers, thought to represent degenerative changes in astrocytes, also are common. Large macrocystic structures filled with proteinaceous fluid and containing a mural nodule are seen in as many as 50% of patients. The walls of these cysts may be highly vascular, leading to occasional instances of spontaneous hemorrhage. Even though this tumor often displays features otherwise associated with malignant behavior, such as nuclear atypia and focal leptomeningeal invasion, it rarely behaves in other than a benign fashion. [143,351] Although most tumors remain confined to the cerebellum, direct extension through the cerebellar peduncles to involve the brainstem may occur. On rare occasions, both pilocytic and fibrillary cerebral astrocytomas have demonstrated neuraxial dissemination or late malignant transformation, a behavior that belies their typically low-grade histologic features.[464] In contrast to supratentorial astrocytic tumors, high-grade astrocytomas, such as glioblastoma, are uncommon in the cerebellum.

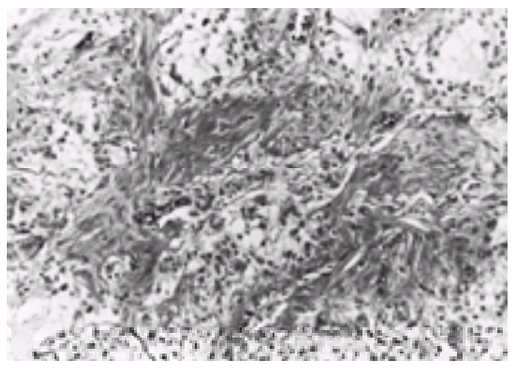


**FIGURE 27-15.** Typical biphasic pattern of a pilocytic astrocytoma. Note the dense, relatively anuclear fibrillar areas alternating with looser honeycombed fields. (Hematoxylin and eosin, ×250.)

The second variety of cerebellar astrocytoma is the diffuse or fibrillary astrocytoma, which accounts for 15% of cerebellar astrocytomas and is similar to the diffuse LGA of the cerebral hemispheres. This tumor is more densely cellular, lacks the microcysts and Rosenthal fibers common to the pilocytic tumors, is more widely infiltrative, and is more likely to undergo anaplastic change than is its counterpart. [351]

### Prognostic Considerations

Published reports of the management of LGA in children are complex, and the identification of consistent prognostic factors is difficult. Most reports include adult and pediatric cases, tumors from all sites, and patients treated over several decades, during which time diagnostic and therapeutic techniques have changed. The very good outcome reported by most authors and the indolent natural history of these tumors confounds analysis as well. Even so, certain factors consistently emerge in analyses but with inconsistent results. Complete resection of tumor seems most important for achieving prolonged disease-free survival in most, but not all,

series.[200,445,446,449,451,467,468] After a radical resection (i.e., greater than 90% of tumor resected), 5-year PFS rates for cerebral astrocytomas exceed 75% [145,467,469] (Table 27-10) versus less than 50% after incomplete resections.[145,146] However, the amenability of these tumors to second surgical explorations results in survival rates that may not differ from tumors completely resected at diagnosis.

| Treatment | Survival in yr (%) | | | Study |
|---|---|---|---|---|
| | 5 | 7 | 10 | |
| Complete resection | 76-100 | 86<br>60 (d) | 69-100 | 200, 467, 477 |
| Incomplete resection | 62 | — | 67-87 | 200, 478 |
| Incomplete resection plus irradiation | 80<br>58-93 (d) | —<br>77 (d) | 67-94<br>67 (d) | 200, 478 |

d, diencephalic.

**TABLE 27-10. SURVIVAL RATE ACCORDING TO THERAPY IN LOW-GRADE ASTROCYTOMAS**

The independent influence of the histology of tumors—generally pilocytic versus nonpilocytic—is controversial. Some reports support superior survival rates with pilocytic histology,[449,470,471,472,473] and [474] and others report equivalent outcomes for pilocytic and nonpilocytic tumors.[146,213,451,454] Related somewhat to histology and to the degree of resection is the invasiveness of the tumor into surrounding structures and amount of tumor residual. In particular, pilocytic and oligodendroglial tumors, which often are well circumscribed, appear more often to be amenable to extensive resection and more likely than fibrillary astrocytomas to have a favorable prognosis.[146,200,462,467,475] The tendency toward invasiveness among nonpilocytic astrocytomas is in keeping with the less favorable prognosis that some have reported for these tumors.[470,472] However, separating the effects of histology and the extent of resection is difficult. Invasion into the brainstem is a primary factor limiting complete resection of cerebellar LGA[445,449,476] and is an independent prognostic factor in one of these series.[476] Volume of tumor residual emerged in another series as the most important predictor of cerebellar LGA progression, emphasizing the importance of maximal tumor resection.[449]

Although the benefit of radiotherapy is not entirely clear, higher-dose radiation (i.e., greater than 53 Gy) significantly improved length of survival in Shaw's series.[474] Young age is noted consistently to increase the risk of progressive disease.[445,451,465]

The ongoing CCG/POG natural history study, which has accumulated more than 700 cases of low-grade gliomas with centrally reviewed pathologic material and radiologic studies, may address more conclusively the independent contributions to prognosis of surgery, histology, and tumor location.

## Treatment

### *Surgery*

The goals of surgery for low-grade gliomas are to obtain tissue for diagnosis and to remove as much tumor as is safely feasible. Current operative mortality rates are less than 1%; morbidity depends largely on tumor location and is highest in diencephalic tumors, in which the incidence of hemiparesis or visual field deficits may be 10% to 20%. Although gross total excisions are possible in 40% to 80% of hemispheric tumors, fewer than 40% of diencephalic tumors are similarly resectable.[479,480] However, the use of microsurgical techniques has led to a resection rate of up to 90% in some diencephalic tumors in a single-institution study.[481]

#### Cerebral Hemisphere Gliomas

Although complete resection usually is the operative goal for cerebral hemisphere low-grade gliomas, its achievement may be difficult for nonpilocytic tumors, which rarely have a distinct tumor-brain interface. Because malignant degeneration of nonirradiated cerebral low-grade gliomas is uncommon, lesions that progress after an initial operation often are amenable to repeat resection. This possibility contrasts with the situation in adults, in which the majority of low-grade gliomas exhibits malignant features at the time of progression.[482]

For cortical and many subcortical tumors, the surgical approach follows the most direct trajectory to the lesion. In recent years, a variety of physiologic monitoring tools have been implemented to facilitate extensive resection of gliomas in “eloquent” regions of the brain. These include functional MRI and direct cortical mapping using strip, grid, and bipolar contact electrodes. Although it is difficult to prove that any or all of these modalities are essential to achieving tumor resection, they clearly increase the comfort level of a surgeon attempting resection of a tumor in, or adjacent to, a functionally critical region of the cortex. Similarly, investigators have debated the need for intraoperative ECOG (direct cortical electroencephalographic monitoring) in children with tumor-associated epilepsy; 75% of patients whose tumor resections are performed without ECOG are free of seizures postoperatively, versus 85% of those with ECOG.[212,483,484] If used, ECOG is likely to be of most value in treating those patients with long-standing or severe seizure disorders.

For deep or poorly circumscribed superficial lesions, imaging-based neuro-navigation and intraoperative imaging using ultrasonography and MRI guidance are helpful for planning an approach to the tumor that avoids traversing critical regions and for monitoring the progress of the resection. These strategies are particularly valuable for lesions that arise from or extend into the thalamus and basal ganglia.

The approach to deep subcortical lesions also is influenced substantially by the predominant direction of tumor growth. Lesions deep within the temporal lobe or temporal horn of the lateral ventricle are approached through a corticotomy in the middle temporal gyrus or sulcus. Lesions that grow medially and encroach on or expand within the lateral ventricle can be approached transcallosally or transfrontally, through the middle frontal gyrus, whereas tumors that extend laterally in the nondominant hemisphere may be approached through the insula after the sylvian fissure has been opened. Laterally extending lesions within the dominant hemisphere and tumors that arise more posteriorly within the thalamus may be reached using a posterior parietal approach situated behind the sensorimotor cortex and above the angular gyrus or through a posterior incision in the middle temporal gyrus. Such lesions can be reached also via an occipital transtentorial trajectory via an opening in the pulvinar. Finally, tumors that project anteriorly and laterally can be reached from a paramedian frontal trajectory, provided that care is taken to avoid injury to the motor pathways.

#### Cerebellar Gliomas

As with supratentorial hemispheric gliomas, a close correlation exists between the extent of resection and outcome in cerebellar gliomas. As shown in Table 27-11, complete tumor excision is associated with improved long-term and disease-free survival. Because the survival of patients with GTRs is as high as 90% for up to 30 years, aggressive attempts at resection are warranted, except when the tumor has invaded the cerebellar peduncles or brainstem.[143,448,465,470,478] The operative approach is similar to that described earlier for resection of a medulloblastoma. Data suggest that as few as 36% of patients with subtotal resections remain free of relapse at 6 years, and actuarial survivals similarly may decline with longer follow-up times of 10 to 20 years.[143,213,448,466,485] Thus, the appearance of resectable residual tumor on a postoperative scan frequently is an indication for reoperation.

| Treatment | Survival in yr (%) | | Reference |
|---|---|---|---|
| | 5 | 10 | |
| Complete resection | 76 (n = 64) | — | 143 |
| | 100 (n = 18) | — | 448 |
| | 100 (n = 9) | 100 (n = 9) | 478 |
| | — | 9 (n = 42) | 465 |
| Subtotal resection without radiotherapy | 56 (n = 34) | — | 143 |
| | 36 (n = 14)[a] | — | 448 |
| | 65 (n = 3) | 33 (n = 3) | 448 |
| | 65 (n = 16) | 65 (n = 16) | 465 |
| | — | 69 (n = 13)[b] | 485 |
| Radiotherapy | 92 (n = 12) | 72 (n = 12) | 478 |
| | 70 (n = 30) | 63 (n = 30) | 384 |
| | 88 (n = 30)[a] | 76 (n = 30)[a] | 200 |

[a]Disease-free survival.
[b]Two- to 31-year follow-up.

**TABLE 27-11. SURVIVAL WITH AND WITHOUT RADIOTHERAPY IN COMPLETELY AND INCOMPLETELY RESECTED CEREBELLAR ASTROCYTOMAS**

Most pilocytic astrocytomas have a distinct margin and can be separated from adjacent cerebellum with reasonable safety; currently, as many as 90% of patients have GTRs with an operative mortality rate of less than 1%.[143,470,472,485,486] Although complete resection is feasible also for the majority of nonpilocytic astrocytomas, its achievement is more difficult because these lesions rarely are as well circumscribed as the pilocytic tumors.

### Gangliogliomas and Other Benign Neuroepithelial Tumors

The approach to gangliogliomas is similar to that for other low-grade gliomas. Complete resection of cerebral gangliogliomas may be associated with survival rates in excess of 90% at 10 years, and recurrences are infrequent. The recurrence rate is substantially higher for deep-seated lesions, such as those within the diencephalon, because of the difficulties in achieving a GTR. However, even after partial resection, long-term progression-free intervals may ensue. The response of the desmoplastic mixed neuronal-glial tumors appears similar; GTRs generally are curative, and incomplete removals have been associated with local recurrence or tumor progression.[150,487,488,489,490 and 491]

#### *Radiotherapy*

The role of radiotherapy in patients with low-grade gliomas depends on the degree of tumor resection. As shown in Table 27-10, patients who have had completely resected tumors have survival rates up to 100% and do not require postoperative irradiation. However, the utility of radiotherapy in patients with incomplete resections is debated. Studies from which data can be abstracted frequently include both adult and pediatric patients who are accrued over several decades and in whom all low-grade tumors, regardless of site or histology, are combined. An additional caveat with respect to these retrospective studies is that they probably were biased toward including patients with a less favorable prognosis in those groups who received radiotherapy. Nonetheless, these reports suggest that radiotherapy may improve survival in these patients. In one review, PFS of children who underwent subtotal tumor resection was improved by the administration of radiotherapy, although overall survival was unaffected.[200] Investigators have suggested that irradiation may be associated with late anaplastic transformation of low-grade tumors. Noting the foregoing and recognizing the improvements in neurosurgical techniques, approaches to patients with incompletely resected tumors may reasonably include observation, with further surgery when it is a feasible option, chemotherapy (addressed later), and no immediate adjuvant irradiation.

For patients with progressive or recurrent cerebral low-grade gliomas, evidence suggests that whole-brain therapy is unnecessary and that local treatment is sufficient. Because 90% of recurrences are local, even after prior limited-field irradiation (e.g., conformal therapy), irradiation of the area of the tumor plus a 2-cm margin is adequate. Both interstitial and stereotactic irradiation have been advocated for some selected newly diagnosed and recurrent tumors; however, their ultimate utility remains to be determined.

The role of radiotherapy in incompletely resected cerebellar tumors is unclear. All reported comparisons involve retrospective reviews of patients accumulated over periods as long as 40 years, most of which patients were not treated uniformly with respect to dose and type of radiation. As shown in Table 27-11, some single-institution studies suggest that radiotherapy may improve the survival of these patients. The numbers of patients in these studies are low, however, and more recent studies with larger numbers of patients are unable to demonstrate a significant advantage for such treatment. As suggested for other low-grade gliomas, patients with incomplete tumor resections of cerebellar tumors should be considered for further surgery, and radiotherapy should be reserved for patients with residual or progressive tumor. Intracavitary $^{32}P$ may be useful in some patients with recurrent macrocystic tumors. Although limited-field conventional radiotherapy is effective in producing long-term disease control, single-fraction stereotactic radiotherapy also has produced encouraging initial results.

Irradiation has been proposed for patients who have gangliogliomas and have undergone incomplete resections or disease recurrence. However, the reported numbers of patients treated with radiotherapy are insufficient to estimate reliably the long-term utility of such treatment. Long periods free of tumor progression may follow incomplete resection alone.

#### *Chemotherapy*

LGA is primarily a surgical disease. When tumors recur after complete or incomplete resection, reoperation generally is indicated, and subsequent resection may lead again to prolonged disease-free status. However, aggressive primary or secondary surgery may be unsafe for tumors in deep locations or eloquent structures. In those situations, in cases in which deferring radiotherapy has been desirable, and in children whose tumors have progressed after irradiation, chemotherapy has been explored. Numerous single-agent and combination regimens have been reported in series involving low-grade gliomas of all sites in patients from a few months of age through adolescence.[492,493,494,495,496,497,498,499,500 and 501] Regimens have been based largely on classic alkylators, nitrosourea, or platinum. The most common responses are stable disease and partial remission. Complete remissions are rare, and progressive disease occurs in a subset of patients in many series. Data regarding cyclophosphamide are conflicting. Benefit was seen after a short course of high-dose cyclophosphamide in all four patients with disseminated pilocytic astrocytoma reported by McCowage et al.[494] In a classic phase II study for patients with progressive LGA, however, activity was so low that the study was closed early.[502]

The most promising chemotherapy data come from two reports. In the first, carboplatin and vincristine were administered to 73 patients with newly diagnosed, progressive LGA. Most tumors were in the diencephalon, and the mean patient age was 3 years. Radiographic responses were seen in 56% of patients. Three-year PFS rates were 68%. Histologic type of astrocytoma, location of tumor, or maximum response to chemotherapy did not correlate with the duration of disease control. The only significant factor was age: Children 5 years old and younger had a significantly higher rate of 3-year PFS (74%) as compared with children older than 5 (39%; $p < .01$).[496] The other promising reports come from a chemotherapeutic regimen based on experimental *in vitro* laboratory data showing that chemical interactions could increase nitrosourea cytotoxicity and possibly overcome resistance to that agent. The regimen—6-thioguanine, procarbazine, dibromodulcitol, CCNU, and vincristine—resulted in no complete responses, a definite degree of tumor reduction in 36% of patients, and stable disease in 59%; 5-year survival was 78%.[501] In contrast to the carboplatin-vincristine data, older age was the only factor that improved survival significantly. These two regimens form the basis of an ongoing study within the COG for patients with progressive low-grade glioma.

Although chemotherapy appears to be a viable treatment option for children in whom either aggressive surgery or radiotherapy is inadvisable, the natural history of LGAs, characterized by recurrence or progression rates many years after diagnosis or after irradiation, will require up to 20 years of follow-up to determine its long-term benefit. In the shorter run, comparative activity of various agents will be determined. Biologic factors may guide chemotherapy further in the future.

## TUMORS OF THE OPTIC PATHWAY

### Demography

Optic pathway tumors (OPTs) generally are those tumors that arise in the optic nerves, chiasm, and hypothalamus and may extend from these sites to adjacent brain structures. Together, they represent approximately 5% of pediatric intracranial tumors.[503,504] Nearly two-thirds of OPTs are diagnosed in the first 5 years of life.[504] Seventy-five percent of OPTs will become symptomatic in the first decade of life, and 90% will become so before the age of 20.[505] Boys and girls are affected equally. OPTs are prevalent in patients with NF-1. The incidence of NF-1 in reported series of OPT ranges as high as 28%,[506] and as many as 70% of OPTs may be

associated with NF-1.[168] The sites of OPT involvement appear to differ in patients with and without NF-1. Unilateral or bilateral optic nerve involvement alone is seen almost exclusively in patients with NF-1, whereas chiasmal involvement is significantly more common in patients without NF-1. [506,507]

### Pathology and Patterns of Spread

Histologically, OPTs usually are low-grade pilocytic and occasionally fibrillary astrocytomas with microscopic features virtually identical to those of the classic cerebellar astrocytoma and other midline pilocytic tumors. Malignant degeneration is rare. [508] Although these tumors usually are confined to the structures of the visual pathways and extend in contiguity along them (Fig. 27-16), they may extend also into the frontal lobes, hypothalamus, thalamus, and other midline structures. Such events are more frequent in chiasmal tumors. Overall, tumor growth is slow, although alternating periods of clinical progression and stability suggest an erratic growth pattern.[508,509,510,511,512 and 513]

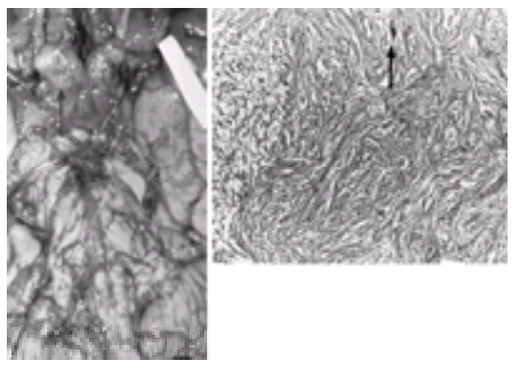


**FIGURE 27-16. A:** Diffuse infiltrating glioma of the right optic nerve (*arrow*). Note the diffuse enlargement of the nerve and absence of a separate mass lesion. **B:** Optic nerve glioma formed by elongated, swirling piloid processes of astrocytes, the nuclei of which are inconspicuous. Note the plump Rosenthal fiber (*arrow*). (Hematoxylin and eosin, ×250.)

### Prognostic Considerations

Three factors appear to have prognostic importance for the outcome of OPTs, although reports are not always consistent. The presence of NF-1 generally is associated with more indolent disease, reflected in longer times to disease progression and higher rates of PFS and survival. [137,477,507,514,515,516,517 and 518] For example, 15-year relapse-free survival in Jenkin's report[518] was 84% for patients with NF-1 and 47% for those without ($p = .0007$). In Imes's series,[517] patients with NF-1 survived their OPT better than did those without NF-1 but died of other causes, including other intracranial tumors and complications of NF-1 resulting in a survival not different from nonneurofibromatosis patients. In other series, NF-1 does not offer a protective effect. [519,520] Tumors involving the chiasm and hypothalamus have a worse prognosis in most (but not all) series.[508,516,518,521] Finally, as with most other CNS tumors, youngest children, generally younger than 3 to 5 years, do worse than do their older counterparts.[516,520,522]

### Treatment

#### *Surgery*

Because of the variability in the growth properties of OPTs, diverse approaches to management have been advocated in different clinical situations and in different institutions. In children with NF-1, the etiology of the lesion rarely is in question. Because the tumor usually exhibits diffuse involvement of the chiasm and nerves, it is not amenable to extensive resection. Often, it is biologically indolent, resection generally is not pursued, [23,523,524], and adjuvant therapy is initiated empirically.

Lesions that seem particularly well suited to radical excision are those that involve only a single optic nerve and produce progressive, disfiguring proptosis or blindness (or both) and those that grow exophytically from the optic chiasm and produce significant mass effect or hydrocephalus. [510,511,522,524] For isolated optic nerve gliomas, which are fairly uncommon, the tumor can be removed with preservation of the globe. In such cases, the resected segment of the optic nerve should be as long as possible, preferably extending close to the chiasm, to diminish the risk of local tumor recurrence. [511] Ruling out the diagnosis of NF-1 before embarking on surgery is essential, because such children commonly exhibit widespread involvement of the optic pathways and may have long-term stabilization of vision without aggressive intervention. For exophytic chiasmatic-hypothalamic tumors, resection often is pursued to relieve obstructive hydrocephalus, to reduce local mass effect, and to establish a tissue diagnosis. For lesions amenable to resection, the tumor is approached via a subfrontal, trans-sylvian, or transcallosal exposure, depending on the pattern of tumor growth. Although a complete resection is not feasible because these lesions infiltrate the optic chiasm or hypothalamus (or both), substantial symptomatic improvement sometimes can be achieved.[522,524] In occasional cases, removal of a significant portion of the tumor may stabilize the disease and delay the need for additional therapy.[522]

Open biopsy also is pursued sometimes for lesions involving the chiasm in which the histologic diagnosis is uncertain, before instituting further therapy. This is especially the case in children without neurofibromatosis or those with isolated chiasmatic-hypothalamic lesions without contiguous optic nerve or optic tract involvement. However, this procedure may further compromise vision in a significant percentage of patients. [129,525,526] Alternatively, some neurosurgeons prefer to perform a stereotactic biopsy and treat with chemotherapy, reserving open resection for lesions that fail to respond or subsequently progress. Although aggressive surgery may be of potential benefit in some patients with large, progressive lesions, it may be associated with significant morbidity, particularly in the youngest patients, and does not convincingly improve survival in comparison to more limited open or stereotactic biopsy and adjuvant therapy. [522,527]

#### *Radiotherapy*

Radiotherapy has been shown to stop the progression of these gliomas, to stabilize and preserve vision and, occasionally, to improve vision. The treatment decision today, however, is dependent on several factors: the degree of vision loss at diagnosis, evidence of progression of the tumor by visual decline or as documented on imaging studies, the age of the patient, and the presence or absence of NF-1. For young patients, the definition of which is not standard but could include children younger than 10 years, primary chemotherapy is reasonable if therapy is in fact indicated. NF-1 is associated with a vascular phenomenon called *moyamoya syndrome*, which appears to be exacerbated by radiotherapy. One should, therefore, be more conservative in recommending radiotherapy as the first treatment in children with NF-1. If irradiation is needed, the usual dose is 45 to 50 Gy, and a conformal technique generally is preferred.

Patients with chiasmal-hypothalamic gliomas have a worse prognosis than those with optic nerve gliomas. In addition, such tumors occasionally may spread along the leptomeningeal space. Although primary chemotherapy is a reasonable option, a significant number of children treated with primary chemotherapy eventually will require radiotherapy for treatment of tumor progression. Ten-year survivals after radiotherapy have been reported as 40% to 93%. [137,514,518,528,529]

#### *Chemotherapy*

The optic glioma chemotherapy experience is greatest for children who are younger than 3 years and for whom delay of radiotherapy is desirable for avoidance of long-term neuropsychological and neuroendocrine effects. Packer's regimen of vincristine and actinomycin-D was the first to defer radiotherapy successfully for children who are younger than 5 and have progressive chiasmatic and hypothalamic gliomas. At a median of 4 years' follow-up, 62.5% of the patients remained free of progressive disease and had not received radiotherapy. [530] By 7 years, however, only one-third of patients were free from progression. Packer also tested a carboplatin and vincristine regimen described for low-grade gliomas. [496] Recently, the POG reported the results of its phase II study of carboplatin for children who are younger than 5 years of age and have progressive OPT. Carboplatin was administered alone every 4 weeks at a dose of 560 mg/m $^2$. After two courses, patients were evaluated, and those with stable disease or better were continued on therapy for 18 months or until disease progression. Of 50 eligible children, including 21 with NF-1, 39 (78%) had stable disease or better, and 34 completed therapy. Six children, of whom only one had NF-1, died of disease. [531] These reports have established

carboplatin as an effective chemotherapeutic agent for OPT.

Petronio et al.[532] reported improvement or stable disease in 15 of 18 patients with progressive OPT using the five-drug regimen of 6-thioguanine, procarbazine, dibromodulcitol, CCNU, and vincristine. Of the 15 patients, 11 had a greater than 50% decrease in their tumor mass. Those children whose tumors progressed on or after completion of chemotherapy were treated successfully with radiotherapy. Using chemotherapy, vision was stabilized in 14 of 18 patients and improved in 2. The ongoing COG study for progressive low-grade gliomas includes children with OPT and is a randomization between carboplatin-vincristine and a modified 6-thioguanine, procarbazine, cisplatin, and vincristine regimen. Future studies will examine the efficacy of other agents for OPT. Already, reports of limited numbers of patients have identified cisplatin and vincristine, tamoxifen and carboplatin, and oral etoposide as having activity against progressive OPT. [533,534 and 535]

Most reports of chemotherapy for low-grade gliomas include patients whose tumors arose from the optic pathway. (See Supratentorial Low-Grade Gliomas for additional chemotherapy data.)

## SUPRATENTORIAL HIGH-GRADE GLIOMAS

### Demography

Anaplastic astrocytoma, glioblastoma multiforme, and mixed glial tumors with a preponderance of malignant astrocytic elements collectively compose malignant or high-grade astrocytic tumors in children. These tumors represent 7% to 11% of childhood CNS tumors. When primary brainstem tumors are excluded, combined series suggest that approximately 25% of malignant or high-grade astrocytic tumors occur in deep midline structures of the cerebrum, not more than 15% occur in the posterior fossa, and the majority occurs in the cerebral hemispheres.[190,191,536,537 and 538] The median age at diagnosis is 9 to 10 years, and the male-female ratio is near unity.[190,537,539]

### Pathology and Patterns of Spread

Various classification schemes have been applied to astrocytic tumors. High-grade lesions, unlike their low-grade counterparts, generally are characterized by the presence of several histologic features of malignancy that include hypercellularity, cytologic and nuclear atypia, mitoses, necrosis, endothelial proliferation, and other anaplastic features; lesions with these features may be termed *malignant* or *high-grade* gliomas. The most common malignant glial neoplasms are the high-grade astrocytomas, such as the anaplastic astrocytoma (Fig. 27-17) and glioblastoma multiforme (Fig. 27-18), which may alternatively be termed *grade III* and *grade IV* astrocytomas, respectively.[95] Similarly, the term *high-grade* or *anaplastic* may be used to describe other, less common glial neoplasms, such as oligodendroglioma, ganglioglioma, or mixed astrocytic-oligodendroglial neoplasms.

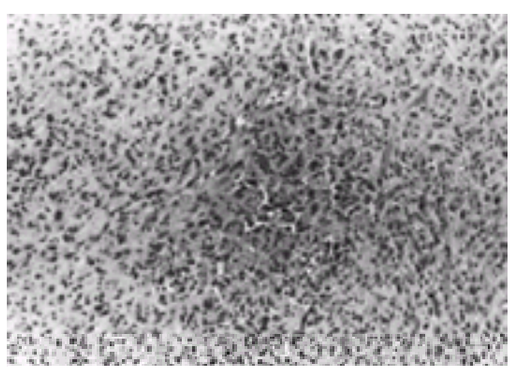


**FIGURE 27-17.** Highly cellular tumor composed of anaplastic astrocytes. A few vessels with compressed lumina are formed by swollen epithelial cells. (Hematoxylin and eosin, ×250.)

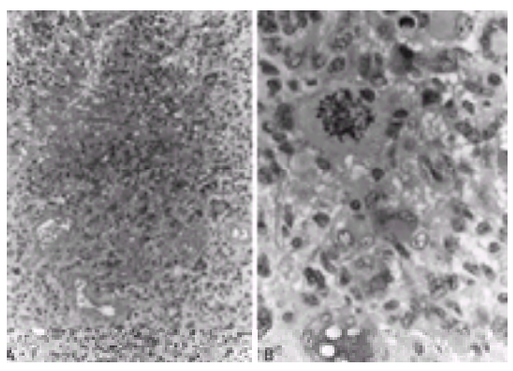


**FIGURE 27-18. A:** Typical field in glioblastoma multiforme showing pseudopalisading ( *upper left*), neovascularity, nuclear anaplasia, and multinucleate giant cells (*lower right*). (Hematoxylin and eosin, ×250.) **B:** Higher magnification of a different field from the same tumor showing cellular anaplasia, multinucleate cells, and bizarre mitosis (*upper left corner*). (Hematoxylin and eosin, ×400.)

The high-grade astrocytomas are clinically aggressive, regionally invasive, and capable of extraneural dissemination to lung, lymph nodes, liver, and bone, particularly in adults. In children, these and the other malignant gliomas occur most commonly in the cerebral hemispheres, in contrast to the more frequent cerebellar and deep midline locations for low-grade tumors. Although the rapid growth and effacement of normal tissue produced by high-grade tumors may produce what appears to be a well-demarcated tumor, microscopical study frequently demonstrates extension for up to several centimeters beyond this margin. Distant neuraxial dissemination, once considered unusual, has been demonstrated in as many as 25% to 50% of high-grade astrocytomas, both at diagnosis and post mortem in carefully evaluated series of patients.[538,539 and 540]

High-grade gliomas may have a histologically heterogeneous nature in that areas of low-grade histology commonly are noted in many high-grade tumors, particularly in small biopsies taken from the more superficial areas of tumor. Diagnostic confusion may be reduced by more generous sampling and by directing stereotactic biopsies toward the contrast-enhancing or more central portions of the tumor.

### Prognostic Considerations

Several series conducted by both institutional and cooperative groups noted the prognostic importance of the extent of surgery, regardless of the primary site of tumor.[189,190,191 and 192,536,537,541,542] In these series, outcome is better with complete or near-complete resection of primary disease. For example, in the CCG-945 study, patients who were reported to have a greater than 90% resection of their tumor had a median PFS significantly longer than that in patients with more limited resections. The difference was more notable for patients with grade III tumors (31 versus 12 months) than for those with grade IV tumors (12 versus 8 months) but was significant for both groups.[190] The relationship may be surprising, given the fact that tumor often extends well beyond the identified central component of tumor [543,544 and 545] and that, even after a radiologically confirmed GTR of all contrast-enhancing tumor, extensive residual tumor is known to remain. A caveat worth emphasizing is that factors specific to the tumor, such as the pattern of growth and degree of infiltration, may determine which tumors are amenable to extensive resection. Thus, tumors that are amenable to resection may constitute a group biologically more favorable than those that infiltrate extensively into the surrounding brain. [546] Insights from genomic analyses of these tumors may provide better understanding of these issues.

In several series, site of disease is independently prognostic of outcome as well, with deep midline tumors showing a survival poorer than that of cerebral hemispheric tumors. However, hemispheric tumors are more amenable to radical resection than are midline tumors.[190] Data are conflicting in regard to the impact of gender and age at diagnosis; however, female gender and younger age have been shown by some investigators to affect outcome favorably.[190,192,538,547] The impact of histology—anaplastic astrocytoma versus glioblastoma multiforme—is debated as well. Anaplastic astrocytoma may be favorably prognostic for subsets of patients[190,191 and 192] or not at all.

### Treatment

#### *Surgery*

The surgical techniques employed for tumor resection are similar to those used for supratentorial low-grade gliomas. As with the latter tumors, malignant gliomas often are amenable to reoperation at the time of disease progression, because most lesions recur at the primary site. Although the majority of children succumbs to further disease progression despite additional intervention, attempted re-resection may be warranted in the selected group of tumors that are amenable to gross total or radical subtotal removal, in view of recent reports of long-term survival after extensive resection followed by high-dose chemotherapy.[548]

#### *Radiotherapy*

In combined series of pediatric patients with malignant gliomas, postoperative local or wide-field irradiation to 50 to 60 Gy after incomplete surgical resection leads to median survivals that uncommonly exceed 1.5 years, with only 0% to 30% of patients surviving beyond 3 years.[384,538,539,547,549,550] Median time to progression rarely exceeds 1 year. The addition of single- or multi-agent chemotherapy in phase II and phase III trials along with irradiation has had little or no impact on the overall survival in patients in this group, despite producing response rates as high as 45%.

An objective analysis of the issue of treatment volume has correlated field size with CT and MRI scans and whole-brain-mount pathologic sections.[545] This study suggests that limited-field irradiation with a 2- to 4-cm margin around the area of “edema” defined by imaging should be adequate to cover the tumor in most cases and should produce survival rates equivalent to those seen after whole-brain treatment. Similar conclusions have been reached in clinical trials in which little or no difference in survival was evident between whole-brain and limited-field treatment volumes. Modern imaging studies, such as PET and MRS, may further improve the targeting of subclinical disease. The corpus callosum and the subependymal pathway along the ventricular wall are the potential routes of spread within the brain and should be included in the treatment volume if the gross tumor involves any part of these regions.

Noting that most recurrences of malignant gliomas are local and occur within the initial treatment volume, attempts to improve local control have focused on techniques that employ locally enhanced radiation doses. Although some reports have suggested that “boosting” the local tumor dose using brachytherapy, offering up to 60 Gy additional dose, may be associated with some increased survival, this advantage may be the result of selection bias and the accrual of patients with smaller tumors and prognostic features better than those receiving standard treatment.[551] Additionally, a substantial incidence (40% to 56%) of radionecrosis is associated with brachytherapy[552,553]; the need for surgical intervention in many such patients suggests that the disadvantages of this technique outweigh its advantages, particularly in patients with anaplastic astrocytoma.

Currently, attention is turning away from brachytherapy and toward the use of stereotactic radiotherapy as a technique to increase local tumor doses. This technique has the advantage of being noninvasive and providing a more focal and homogenous dose that may be associated with a lower incidence of radionecrosis. The benefit of a radiosurgical boost is controversial and is the subject of a current COG trial.

#### *Chemotherapy*

The role of chemotherapy for treatment of high-grade gliomas has yet to be defined clearly. A single prospectively randomized phase III trial was conducted by the CCG between 1976 and 1981. Postoperatively, children who were between the ages of 2 and 21 and had nonbrainstem and non–spinal cord high-grade astrocytomas were assigned randomly to radiotherapy with or without chemotherapy consisting of prednisone, CCNU, and vincristine (PCV). PFS for those children receiving postradiation chemotherapy was significantly higher (46%) than for those who did not receive it (16%; $p = .026$).[537] A significant benefit of chemotherapy was observed in only those patients with glioblastoma; furthermore, chemotherapy did not appear to improve the outcome for any patient whose tumor underwent biopsy only, regardless of histology. For patients who had glioblastoma multiforme and whose tumors were at least partially resected, the addition of chemotherapy significantly improved 5-year event-free and overall survival.[190] The follow-up study by the CCG was again a randomized trial, this time comparing “8-in-1” chemotherapy against PCV. No difference was observed between the two chemotherapy arms, and 5-year PFS rates were 33% and 36% for “8-in-1” chemotherapy and PCV, respectively.[190] Taken together, these studies suggest that the benefit from addition of chemotherapy, when compared to surgery and radiotherapy alone, is modest at best.

In contrast to this experience with anaplastic astrocytoma and glioblastoma multiforme, children in this study, with eligible diagnoses other than anaplastic astrocytoma and glioblastoma multiforme, fared well. Their 5-year PFS and survival rates were 64% and 71%, respectively. Chemotherapy with procarbazine, CCNU, and vincristine has resulted in greater than 50% partial and complete responses in patients with anaplastic oligodendrogliomas and anaplastic mixed gliomas in other studies as well.[554,555] These data suggest that CCNU and vincristine, combined with prednisone or procarbazine and in addition to radiotherapy, probably is the chemotherapeutic regimen of choice for children with these tumors.

Given the poor outcome for children with most high-grade gliomas, numerous single-agent phase II studies have been conducted on cisplatin, carboplatin, CCNU, procarbazine, cyclophosphamide, ifosfamide, etoposide, and topotecan. The data for CCNU and cyclophosphamide have shown the most promise.[498,547,556] To determine the contribution of procarbazine to the results of combination chemotherapeutic regimens for high-grade gliomas, the POG recently completed a phase II evaluation of that agent for patients with newly diagnosed high-grade glioma demonstrating measurable disease. Of 12 evaluable patients, none had a complete response, and only 1 patient had a partial response.[557] Topotecan has been evaluated similarly and was found to have little activity against high-grade gliomas.[558] Initial experience with irinotecan for adults with high-grade gliomas has been reported in abstract form; pediatric studies are ongoing. Temozolomide has generated excitement in adult studies and is under investigation by the COG as well. Combination regimens of BCNU with cisplatin and cyclophosphamide with etoposide have also been evaluated by the POG. Although activity was demonstrated, superiority over CCNU and vincristine has not been determined.[559] Myeloablative chemotherapeutic regimens have shown activity against bulk residual disease but have not demonstrated convincing superiority over standard therapy.[241,243,548,560,561] Clearly, new agents and new approaches to therapy are needed.

## BRAINSTEM GLIOMAS

### Demography

Tumors arising in the midbrain, pons, and medulla oblongata now account for approximately 20% of all CNS tumors among children younger than 15 years.[6] This apparent rise from 10% in the late 1970s does not reflect a truly increased incidence. Instead, this jump stems from the advent of MRI and increased detection of focal brainstem tumors, subsequently confirmed microscopically as low-grade gliomas. The median age of occurrence for all brainstem gliomas is 6 to 7 years.[142] The male-female ratio is near unity. Brainstem tumors are noted to be increasingly frequent among patients with NF-1.[562,563]

### Pathology and Classification

The term *brainstem glioma* is an imprecise descriptor suggesting that all these tumors behave in the same way. As a result of advances in neuroimaging over the last two decades, a plethora of terms emerged to subclassify brainstem gliomas (sometimes confusingly) by site of origin or imaging features. Terms used have included *midbrain tumor*,[564,565 and 566] *tectal glioma*,[567,568,569,570 and 571] *pontine glioma*,[566,568,572] *focal medullary tumor*,[568,573,574] *cervicomedullary tumor*,[573,575,576,577,578 and 579] *diffuse glioma*,[566,568,573,575,576,578] *intrinsic glioma*,[572,575,578,580] *pencil glioma*,[581] *dorsal exophytic brainstem tumor*,[568,573,576,578,580,582,583 and 584] *focal glioma*,[566,574,575,578,580,585] and *cystic glioma*.[580,586] Brainstem tumors are sometimes also subcategorized by pathology, as either low-grade (benign) or high-grade (malignant) gliomas.[580,587,588 and 589] However, brainstem gliomas can be parsimoniously and better biologically classified as diffusely infiltrative brainstem gliomas and focal brainstem tumors, categories

that combine tumor location and histology.[142]

*Diffusely infiltrative brainstem gliomas* are the classic brainstem tumor having a poor prognosis. Most arise in the pons, particularly the ventral aspect, and cause diffuse enlargement of that structure (Fig. 27-19). Engulfment of the basilar artery by tumor is specific for the diagnosis of diffusely infiltrative brainstem glioma but is not seen in all cases (Fig. 27-19B).[142] Axial or exophytic growth is seen in at least two-thirds of cases.[566] Neoplastic infiltration extending into the midbrain, cerebral peduncle, cerebellum, or medulla is exceedingly common. These tumors are generally fibrillary astrocytomas by histology, sometimes well-differentiated, WHO grade II or, more often, high-grade anaplastic astrocytoma (WHO grade III) or glioblastoma multiforme (WHO grade IV). [142] These diffuse gliomas may occasionally show disseminated neuraxis spread.[590,591]

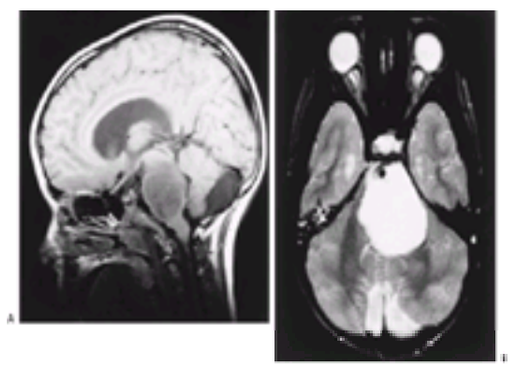


**FIGURE 27-19.** Typical magnetic resonance imaging findings in a diffusely infiltrating brainstem glioma. **A:** T1-weighted sagittal view shows diffuse, fusiform enlargement of the pons with tumor spread superiorly and inferiorly. **B:** T2-weighted transverse view shows engulfment of the basilar artery.

*Focal brainstem tumors* are discrete, well-circumscribed tumors without evidence of infiltration and without edema. These tumors may occur in any level in the brainstem but are most frequently seen in the midbrain or medulla rather than the ventral pons ( Fig. 27-20). More often, focal tumors are dorsally exophytic to the brainstem, sometimes effacing the fourth ventricle. By pathology, focal brainstem tumors are most commonly pilocytic astrocytomas or, rarely, gangliogliomas, both WHO grade I.[142,583] Sometimes these tumors are cystic.[566]

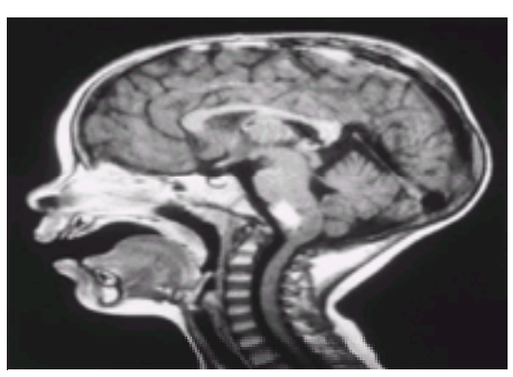


**FIGURE 27-20.** T1-weighted transverse magnetic resonance imaging with gadolinium shows a focal brainstem tumor with acid enhancement at the medulla. Biopsy demonstrated the tumor to be a pilocytic astrocytoma.

Rarely, tumors of histologic types other than gliomas are found at the brainstem. Among infants, the highly malignant AT/RT can arise in the brainstem. [329] Embryonal tumors, or PNETs, can also occur in very young children as localized pontine tumors with an exophytic component and extension beyond the pons, along with a predilection for leptomeningeal dissemination at diagnosis. [592] Hemangioblastoma may arise at the brainstem also, although more commonly during adolescence or adulthood.

**Prognostic Considerations**

Prognosis depends principally on the tumor type but is also influenced by NF-1. Patients with diffusely infiltrative gliomas fare dismally, regardless of therapy. In most series, median survival is less than 1 year, and survival rates at 2 years are lower than 10% to 20%. [593,594] In contrast, the prognosis for patients with focal brainstem tumors may be good after surgery if the tumor is accessible, with or without postoperative radiotherapy, or after radiotherapy alone. Survival for these patients is reported to be between 50% and 100%.[562,566,589] Many patients with small focal tumors in the midbrain, particularly the tectum, may do extremely well after shunting alone and can demonstrate PFS of up to 10 years or more.[567,570,571]

Among patients with NF-1, brainstem gliomas, whether diffuse or focal, display a generally indolent biologic behavior. [562,563] The tumor may stabilize in size or regress without intervention, and survival approximates 90% over 5 years. Thus, intervention should be limited to those lesions that exhibit rapid or unrelenting growth on serial neuroimaging or that produce significant clinical deterioration.

**Treatment**

The choice of treatment depends largely on whether the tumor is a diffusely infiltrative brainstem glioma or a focal brainstem tumor. Even among focal lesions, the site of the tumor affects selection of therapy.

***Surgery***

A major advance in the surgical management of brainstem gliomas followed the recognition that this broad category of tumors encompasses biologically distinct groups that demand individualized therapeutic strategies. At one extreme are the diffuse intrinsic gliomas, which are biologically malignant, highly infiltrative, and not amenable to resection. Although biopsy usually is associated with low morbidity, it no longer is considered necessary because, in the context of a typical clinical presentation and characteristic MRI findings, histologic results do not influence treatment. [174] At the other extreme are the benign intrinsic tectal gliomas, which also are not appropriate for resection because these lesions are extremely indolent and are best treated symptomatically with CSF diversion and observation ( Fig. 27-21).[565,567,570]

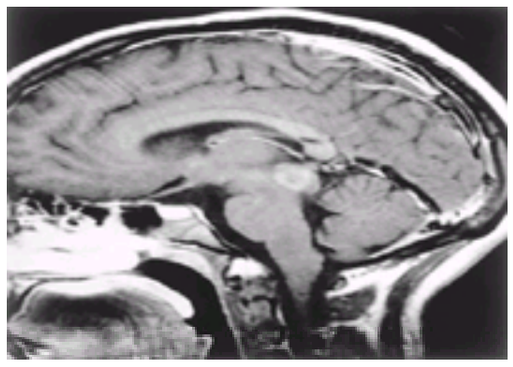


**FIGURE 27-21.** T1-weighted sagittal magnetic resonance imaging with gadolinium of a tectal glioma with minimal contrast enhancement.

In contrast to the preceding groups, and because of improvements in neurosurgical techniques and postoperative care, surgical resection is a reasonable option for focal gliomas that arise at the cervicomedullary junction and for dorsally exophytic gliomas. These lesions are histologically and biologically benign but, unlike tectal gliomas, commonly show gradual enlargement over time. In particular, extensive resection of exophytic tumors, leaving a thin rim of tumor on the surface of the brainstem, frequently achieves long-term PFS without further treatment.[582,583] and [584,589] Some focal intrinsic, cystic, and solid brainstem lesions are likewise surgically resectable, and several authors have suggested that even after only partial resection, many patients require no further postoperative treatment. Although good survival rates have been achieved with surgical resection of symptomatic focal midbrain and medullary lesions, it remains to be determined whether these results represent an improvement over those obtained with stereotactic biopsy and local irradiation, [565,579,595] particularly in view of the potential for significant surgical morbidity from aggressive attempts at resection.[574]

### *Radiotherapy*

Depending on the neurologic status and tumor type of the child with a brainstem glioma, the therapeutic options include observation, radiotherapy, or investigational approaches. Radiotherapy continues to be the mainstay of treatment for most children with diffusely infiltrative brainstem gliomas. Although it rarely produces long-term survival, it frequently affords neurologic palliation, albeit for a relatively short time only.

Because of the poor survival rates that follow standard-dose radiation and the apparent lack of effective chemotherapy, various cooperative groups have studied escalating doses (ranging from 64.8 to 78.0 Gy) of hyperfractionated radiotherapy in a series of phase I and II trials. [596,597,598,599,600,601,602,603,604,605,606] and [607] Collectively, these trials have failed to show any therapeutic benefit from this approach. Median survival was still less than 1 year. However, escalating hyperfractionated radiotherapy did result in increased steroid dependency and clinically significant intralesional necrosis. [599,605,608]

Other treatment approaches have been used for patients with diffusely infiltrative brainstem gliomas. but these too have been largely unsuccessful in achieving cure rates higher than those seen with radiation alone. A CCG phase I and II trial combined hyperfractionated radiotherapy with escalating doses of interferon-b three times weekly. The median time to death was 9 months.[594] A recent POG study randomized patients to either 70.2 Gy hyperfractionated radiotherapy or 54 Gy conventional radiotherapy, with cisplatin infused as a radiosensitizer over 120 hours on weeks 1, 3, and 5 of radiotherapy for both treatment arms. [593] No difference in survival was noted between groups, the 2-year overall survival being just 7%. When the patients who received hyperfractionated radiotherapy with cisplatin were compared to historical controls receiving just 70.2 Gy, the patients who received cisplatin unexpectedly fared slightly less well. [593] In another CCG study, intravenous topotecan used as a radiosensitizer did not appear to alter outcome either, although only limited conclusions about efficacy can be made from this phase I dose escalation trial.[609] Currently, the COG is exploring the effectiveness of gadolinium texaphyrin, another radiosensitizer.

Radiotherapy is used also for patients with focal brainstem lesions that are treated surgically. For those patients with high-grade lesions, radiation is used postoperatively. For those with low-grade lesions, radiotherapy may be delivered postoperatively for symptomatic residual disease, should it exist, or may be deferred until clinical or radiographic evidence of progression is present. [583,584,589] No controlled or prospective trials comparing these two approaches have been performed. If radiation is deferred until progression is seen, additional surgery should be considered as well. As stated earlier, for patients with gliomas confined to the tectum and associated with hydrocephalus, radiotherapy routinely is deferred until there is symptomatic progression after the hydrocephalus has been corrected. [567,569,570] and [571]

### *Chemotherapy*

A role has yet to be established for chemotherapy as standard treatment in the management of patients with brainstem tumors. With focal brainstem gliomas, weekly carboplatin with vincristine has shown activity in a very limited number of patients younger than 5 years. [610]

For diffusely infiltrative pontine gliomas, response rates to various single-agent and multi-agent regimens have been disappointingly low, even when chemotherapy is given as first treatment, before radiotherapy.[325,387,611,612] and [613] Adjuvant chemotherapy using PCV did not improve survival when compared with conventional radiotherapy alone in the only prospective, randomized study that has tested this question. [614] Oral etoposide has been reported to have antitumor activity in a small numbers of patients with recurrent diffuse tumors,[615] and this approach is under investigation in a COG trial that is nearing completion. Approaches that will be investigated in the future will likely include the use of inhibitors of angiogenesis, farnesyl transferase, and epidermal growth factor receptors. As patients with progressive diffusely infiltrative pontine gliomas survive, on average, at least 3 months after their first relapse, they can be candidates for further treatment in clinical trials.[616] Such efforts may delay further disease progression and are requisite for improving the outcome in this disease group.

## PINEAL REGION TUMORS

### Demography

Tumors of the pineal area account for 0.4% to 2.0% of all primary CNS tumors in children. Three principal groups of tumors—germ cell tumors, pineal parenchymal tumors, and astrocytomas—account for most tumors in this location. In combined clinical series, astrocytomas constitute 15%, the pineal parenchymal tumors 17%, and germ cell tumors 40% to 65% of all neoplasms in this area. Of CNS germ cell tumors, two-thirds occur in the pineal region and the remaining one-third in the suprasellar region.[617,618] Pineal parenchymal tumors are more frequent in the first decade of life and have a male-female ratio near unity. Germ cell tumors are most common in the second decade of life or later, have a peak incidence at between 10 and 14 years of age, and are associated with a male-female ratio of at least 2:1 and as high as 9:1. Astrocytomas tend to occur in two separate age groups, 2- to 6-year-old children and 12- to 18-year-old teens, and each group has a 2:1 male-female incidence characteristic of astrocytomas elsewhere in the CNS. [140,619,620] and [621]

### Pathology and Patterns of Spread

### *Pineal Parenchymal Tumors*

Pineoblastoma is a primitive undifferentiated tumor that accounts for approximately 50% of the pineal parenchymal tumors ( Fig. 27-13). Except for its location, this tumor may be indistinguishable from medulloblastoma and is considered by some investigators to be a variant of the PNET. This highly cellular tumor has frequent mitoses and areas of focal necrosis. The occasional presence of Flexner-Wintersteiner rosettes indicates differentiation toward retinoblastoma. [351] Although the histologic appearance of the pineocytoma (Fig. 27-22) may overlap that of the pineoblastoma, the cells generally are larger and have a recognizable relation with blood vessels, and true rosettes rarely are seen. Occasional evidence of astrocytic, neuronal, or ganglion cell differentiation is noted. [351]

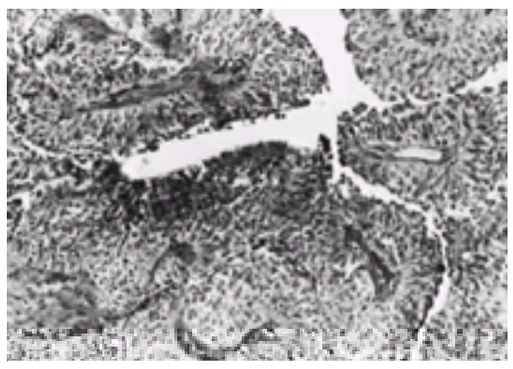


**FIGURE 27-22.** Primary pineal tumor displaying prominent perivascular growth of neoplastic cells characteristic of pineocytoma. Note the papillary pattern. (Hematoxylin and eosin, ×250.)

### *Germ Cell Tumors*

The germ cell tumors include a spectrum of embryonal neoplasms and teratomas believed to be derived from totipotent germ cells that aberrantly migrated to the cranial midline during embryogenesis. The germinoma accounts for approximately 60% of these tumors (Fig. 27-23). Germinomas have a typical two-cell appearance indistinguishable from that of gonadal germinomas and are composed of large, primitive-appearing cells intermixed with smaller lymphoid cells. [140,351,622,623]

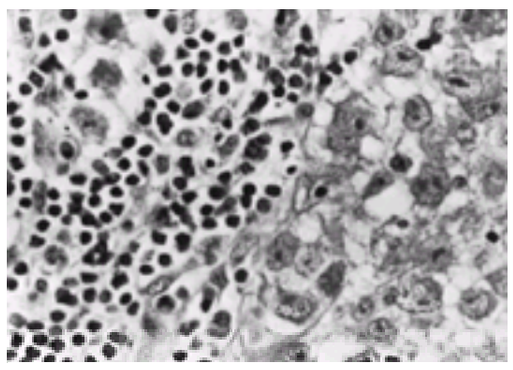


**FIGURE 27-23.** Typical field of germinoma displaying large cells with large nucleoli and focus of lymphocytes ( *right half of field*). (Hematoxylin and eosin, ×400.)

Teratomas and mixed germ cell tumors harboring various mature and immature elements constitute nearly 30% of the pineal nongerminomatous germ cell neoplasms, and the highly malignant embryonal carcinoma, choriocarcinomas, and endodermal sinus tumors constitute the remaining 10%. The histologic appearance of these tumors is identical to that of similar tumors occurring outside the CNS. Teratomas generally remain local, well encapsulated, and noninvasive. However, areas with more primitive germ cell elements may be present and are associated with a more aggressive clinical course that may include neuraxis dissemination. [351,623]

Both contiguous regional extension and distant intra-axial dissemination are common with nonastrocytoma pineal area tumors. The overall incidence of leptomeningeal spread in larger series of patients is approximately 10%, with pineoblastoma and germinoma demonstrating the greatest frequency of such spread.[140,619,622,623] Even pineocytomas, once considered slow-growing, locally infiltrative tumors, may have a high incidence of leptomeningeal seeding. [624,625]

Systemic metastases, although uncommon, may occur with the pineal parenchymal and pineal germ cell tumors. Bone, lung, and lymph nodes are the most common sites of such dissemination.[623] Occasional instances of peritoneal metastasis associated with the use of ventriculoperitoneal shunts have also been noted. [626]

## Prognostic Considerations

Tumor histology has prognostic significance. The germinomas and LGAs have the best overall survival rate and response to treatment, followed by teratomas and pineal parenchymal tumors. Five-year survival rates for intracranial germinomas are as high as 95%, but the remaining nongerminoma germ cell neoplasms have, historically, much poorer survival rates, ranging from 20% to 76%. [627,628 and 629] Disease that has spread regionally, involves the hypothalamus, or has spread along leptomeninges also is associated with a worse prognosis. Although age at diagnosis is variably reported as having prognostic significance, this parameter may not be independent of the finding that very young patients (those younger than 3 years) have a higher incidence of disseminated disease at diagnosis and are frequently treated with reduced-dose irradiation. [140,403,623,630]

## Treatment

### *Surgery*

Because of the diversity in the biologic behavior and response to treatment of different types of pineal area tumors, biopsy is recommended whenever possible to establish a tissue diagnosis, which will help guide subsequent therapy. [140,620,623] One exception is patients with benign intrinsic tectal tumors (discussed earlier) with brainstem gliomas. A second exception is patients with malignant germ cell tumors in which a-fetoprotein or b-human chorionic gonadotropin (or both) is detected at high levels within the blood or CSF, in which case biopsy is considered optional.

Current neurosurgical techniques allow stereotactic or open biopsies in most patients, with morbidity generally limited to transient worsening of prior visual symptoms, although new or permanent losses may occur. The mortality rate is generally less than 2%. Direct visually guided biopsy is preferred by many neurosurgeons because of concern that stereotactic biopsies may injure adjacent deep veins. However, a variety of recent reports have demonstrated that stereotactic biopsy can be performed with acceptable morbidity, provided that a low frontal entry point is used to allow access to the tumor below the internal cerebral veins. [631] This approach also provides CSF for analysis of a-fetoprotein and b-human chorionic gonadotropin, because the biopsy trajectory often traverses the lateral ventricle. Often, it is feasible to achieve CSF diversion using endoscopic third ventriculostomy while the patient is under the same anesthetic; in some instances, the tumor biopsy itself can be accomplished endoscopically. Although these minimally invasive approaches have significant appeal and appear to carry a lower morbidity than conventional approaches, a major concern is the issue of sampling error. As many as 15% of germ cell tumors have mixed histology, which calls attention to the importance of adequate biopsy and of performing CSF and blood marker studies in such patients. [620]

If a stereotactic or endoscopic biopsy is nondiagnostic or equivocal, or if the histology of the lesion suggests that an open surgical resection is likely to be of benefit, as in the case of benign teratoma, tumor removal can be accomplished using one of a variety of operative approaches. An infratentorial supracerebellar approach is used for lesions that exhibit predominant growth below the level of the vein of Galen and basal veins of Rosenthal. A suboccipital transtentorial approach is preferred for larger lesions that extend above the basal veins or down into the rostral fourth ventricle. Both approaches can be accomplished using a prone or modified prone position, which is generally preferred to the sitting position, to minimize the risk of air embolism. After division of the precentral cerebellar vein and surrounding arachnoid, the lesion is subjected to biopsy and then debulked using the ultrasonic aspirator. Great care is taken to avoid injury to the deep veins.

Except for well-encapsulated teratomas, few pineal region tumors are amenable to complete resection, generally because of extensive local or regional disease. Even though subtotal resections are possible for many patients with localized tumors, no evidence indicates that such resections improve outcome. Because many tumors in this area are sensitive to both radiotherapy and chemotherapy, and because aggressive surgery may cause significant morbidity, biopsy alone or limited tumor

debulking to relieve hydrocephalus often is the most prudent approach initially, particularly for patients with germinomas. In addition, a variety of current treatment protocols for nongerminomatous germ cell tumors are employing intensive neoadjuvant chemotherapy after an initial open or stereotactic biopsy, followed by second-look surgery to perform a biopsy or remove areas of residual enhancement. [628,632,633 and 634] In some cases, such reoperations indicate only scar tissue without evidence of viable tumor or foci of mature teratoma that may be amenable to resection, with favorable long-term results.

Pineoblastomas are considered to be PNETs, and their treatment and outcome are comparable to those of high-risk medulloblastomas. However, because these lesions are uncommon, previous studies have contained insufficient numbers of patients to determine conclusively whether extensive resection favorably influences outcome.[401,630]

### *Radiotherapy*

Radiotherapy for pineoblastomas, which generally are not completely resectable, is similar to that for high-risk medulloblastoma—namely, craniospinal irradiation to 36 Gy, followed by additional radiation to the tumor bed to a total dose of 54.0 to 55.8 Gy. For incompletely resected, nondisseminated pineocytomas, local field irradiation to 50 to 54 Gy is appropriate, whereas craniospinal irradiation is indicated for disseminated tumors.

Germinomas are exquisitely radiosensitive tumors. Typically, 10-year survival rates exceeding 90% after operation and radiotherapy can be expected.[617,620,622,623,627,631,635] Patients with germinomas who have evidence of leptomeningeal spread at diagnosis require craniospinal irradiation to 45 to 50 Gy if radiation is the only treatment modality. However, if evidence of leptomeningeal dissemination is absent at diagnosis, the need for routine craniospinal irradiation is controversial. Recent retrospective series have suggested that the cure rates of local field irradiation and craniospinal irradiation are equivalent. [620,621,636,637 and 638] The treatment fields for local field irradiation should include the tumor plus a margin as well as the suprasellar region, including the floor of the third ventricle and the horns of the lateral ventricles, because separate foci of tumor from the primary lesion have been detected in these areas in a few cases, and recurrences at those sites have also been noted if these areas were not irradiated. [637] With conformal techniques, especially IMRT, one can treat the primary tumor to 45 to 50 Gy while simultaneously irradiating the aforementioned suprasellar region to 30 Gy. Recent reports have suggested that a short course of platinum-based, postoperative chemotherapy can produce an excellent tumor response and allow the radiation dose to be reduced to 30 to 40 Gy. [639] No randomized trial has yet compared combined-modality treatment with radiotherapy alone.

In modern studies, craniospinal irradiation is recommended for most nongerminomatous germ cell tumors in conjunction with chemotherapy; the only exception is mature teratoma.[620,625,630,640] For those patients whose tumors lack leptomeningeal spread and who are also treated with chemotherapy, limited data suggest that local irradiation may result in 5-year survival rates equivalent to those achieved with craniospinal irradiation. [641] Unfortunately, even with aggressive therapy, 5-year survival rates are generally less than 50%.[620,627,631,634]

### *Chemotherapy*

Unlike any other CNS malignant tumors, germ cell tumors of the CNS have a model for chemotherapeutic treatment based on histologically similar systemic disease. Outside the CNS, chemotherapeutic regimens based on cisplatin have been very effective in treating disease. This experience, coupled with the mixed response to radiotherapy, prompted the exploration of chemotherapy for CNS germ cell tumors. For patients with germinoma, chemotherapy has been added to decrease deleterious late effects of radiotherapy. For the nongerminomatous tumors, chemotherapy has been added to try to improve cure rates.

There appears to be no question that germinomas are chemotherapy-sensitive tumors. Regimens that use cisplatin, carboplatin, or cyclophosphamide, along with vinblastine or vincristine, bleomycin, and etoposide, are capable of producing complete and partial response rates as high as 90% in newly diagnosed patients.[628,642,643,644,645,646 and 647] In most of these series, patients have had irradiation as part of their treatment; thus, the influence of chemotherapy on survival remains uncertain. Administration of chemotherapy to patients with suprasellar lesions who also commonly have diabetes insipidus may be difficult. Fluid and electrolyte imbalances may result in clinically significant hyponatremia or hypernatremia and dehydration. The use of vasopressin infusions during such chemotherapy may help to avert some of these problems.[648]

With the demonstrated responsiveness of germinomas to chemotherapy, the current debate centers around the optimal balance of chemotherapy and radiotherapy. Recent studies have examined the use of chemotherapy with either reduced-dose radiotherapy or without radiation altogether. [628,646,647] In the largest series wherein chemotherapy was used alone for patients with germinomas, high rates of response were demonstrated to chemotherapy.[628] Despite this, however, 22 of 45 patients ultimately relapsed, a number higher than would have been anticipated after radiotherapy alone. Although a large proportion of the patients who experienced relapse was successfully treated with additional chemotherapy or irradiation, the 2-year overall survival rate for patients with germinoma was only 84%. [628]

Other, smaller studies have examined the use of chemotherapeutic regimens followed by radiotherapy at doses reduced to 30.6 Gy and 40.0 Gy. [646,647] Response rates to chemotherapy have been high, and survival rates in both series are 100%, with median follow-up periods exceeding 32 months. Similar approaches with even lower doses of radiation are being studied by the various cooperative groups. The use of chemotherapy and radiotherapy for intracranial germinomas is rational and effective, although the optimal schedule must be determined. Treatment with chemotherapy alone is not recommended outside of a clinical trial setting.

Similar chemotherapeutic regimens have been applied to nongerminomatous germ cell tumors, with encouraging results. [639,641,649,650,651,652,653 and 654] In these studies, complete and partial response rates to chemotherapy have approached 80%; survival rates several years after diagnosis have similarly ranged from 48% to 80%. Although all patients were treated with radiation as well, the fields varied. Relapse rates appear to be higher in the patients treated with involved fields only. Therefore, craniospinal irradiation for all patients with nongerminomatous germ cell tumors seems advisable.

For a discussion of chemotherapy for pineoblastoma, the reader is referred to the section in which sPNETs are discussed.

## CRANIOPHARYNGIOMA

### Demography

Craniopharyngiomas account for between 6% and 9% of all primary CNS tumors in children. These lesions exhibit a bimodal age distribution, with one peak during childhood at approximately 8 to 10 years of age and a second peak in middle age. [655] This tumor rarely is detected before age 2 years.[447,656] No gender predilection has been noted. Although these lesions are predominantly suprasellar tumors, which involve the pituitary stalk and hypothalamus, they may occur within the sella turcica or third ventricle as well.

### Pathology and Patterns of Spread

Craniopharyngiomas in children are thought to arise predominantly from pharyngeal cell rests left from the embryonic hypophysiopharyngeal duct that connects the infundibular bud with the stomodeum.[657] Although, in adults, these tumors may result from neoplastic transformation of cell rests within the pituitary gland that have undergone squamous metaplasia,[658] this mechanism is less likely in children. [351] Grossly, these tumors are smooth, lobulated masses with both solid and cystic components. The cyst contents may range from gelatinous to viscous oily fluid rich in cholesterol crystals. Rupture of a cyst into the CSF may cause an intense chemical meningitis. Calcification is frequently apparent. Both the cystic lining and the solid portions of the tumor are characterized by squamous epithelium, usually with some evidence of keratinization (Fig. 27-24).

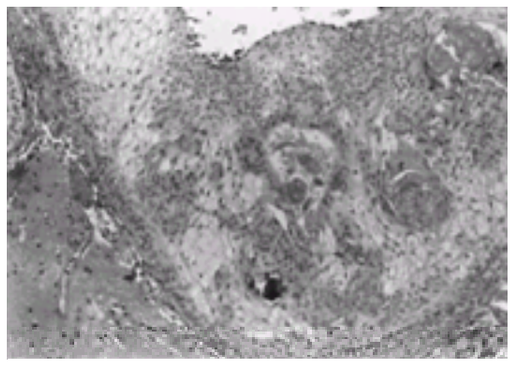


**FIGURE 27-24.** Photomicrograph of typical epithelium found in a craniopharyngioma showing the basisquamous character with incarcerated keratin. Note the honeycombed character of the epithelium in areas. (Hematoxylin and eosin, ×250.)

Although craniopharyngioma is a histologically benign tumor composed of well-differentiated tissue, it may have a malignant clinical course because of its location and its propensity to infiltrate surrounding normal structures. A thick glial layer may encase the tumor, and small islands of epithelial tumor arising within this gliotic scar can extend into adjacent tissues. The tight adherence of this layer to surrounding tissue can make complete resection difficult and hazardous.

### Clinical Presentation

Childhood craniopharyngiomas often manifest with short stature, symptoms of increased ICP, delayed puberty, vision loss, and neurobehavioral abnormalities. Hydrocephalus is observed in approximately 50% of children as a result of obstruction of the third ventricle and the foramen of Monro by superior tumor extension. Because of slow tumor growth, papilledema is less common than optic pallor. Visual field defects of various degrees of severity occur in 50% to 90% of patients, homonymous hemianopsia and bitemporal hemianopsia being the most frequent defects encountered.[656,659] Despite the fact that many children show evidence of vision loss on examination, only approximately 25% present with complaints of visual deterioration.[660]

Various neuroendocrine deficits are present in as many as 90% of patients at diagnosis, decreases in GH and resultant growth delay being the most common findings.[139,659] Diabetes insipidus is seen in fewer than 10% of children in whom craniopharyngiomas are diagnosed in the modern era and, if present in a child with a suprasellar lesion, should raise concern about the possibility of a germ cell tumor or histiocytosis.

### Differential Diagnosis and Evaluation

The differential diagnosis of craniopharyngioma includes intrinsic hypothalamic gliomas, large chiasmal gliomas, Rathke's cleft cyst, and suprasellar germ cell tumors or teratomas. Plain skull radiographs, commonly used before the advent of CT and MRI, often show an enlarged or distorted sella with suprasellar tumor calcification. The CT scan characteristically demonstrates a partially cystic, low-density, contrast-enhancing lesion with calcification.

MRI defines the solid and cystic nature of the tumor, its extent, and its relation to adjacent structures better than does any other modality ( Fig. 27-25).

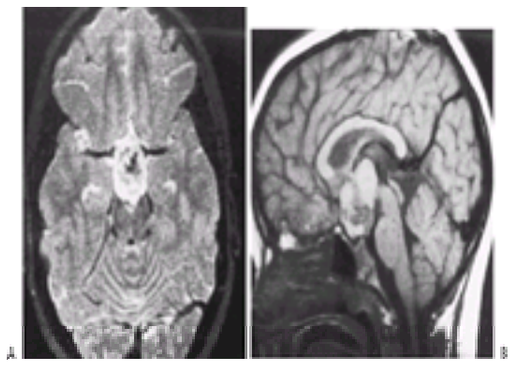


**FIGURE 27-25.** Axial **(A)** and sagittal **(B)** T2-weighted magnetic resonance imaging of mixed-density craniopharyngioma with foci of calcification ( *black*).

Owing to the high incidence of clinical and subclinical neuroendocrine deficits at diagnosis, a thorough evaluation of the hypothalamic-pituitary axis should be undertaken preoperatively. Secondary abnormalities of adrenal function and of the regulation of fluid and electrolyte balance, in particular, can lead to serious perioperative problems if not anticipated. Neuroendocrine evaluations should be repeated postoperatively and periodically thereafter for at least 1 year, because hormonal deficits often increase postoperatively and may take several months to stabilize fully.[661]

### Prognostic Considerations

The extent of tumor resection has been an important factor in series in which the initial treatment has consisted of surgery alone. Patients with totally excised tumors have had considerably better survival rates than those managed by biopsy alone or by subtotal resection.[662] However, several studies have shown that the combination of subtotal resection and radiotherapy achieves survival results that rival those obtained with attempted GTR.[206] The purported prognostic significance of tumor size probably is not an independent variable but rather is related to the ease and extent of resection. Although the data show only a trend, patients with purely cystic lesions appear to survive longer than those with solid or mixed solid and cystic tumors; in addition, children older than 5 years seem to have a better prognosis than do younger patients.[662,663]

### Treatment

#### *Surgery*

Preoperative and perioperative considerations for operation on tumors in the region of the pituitary gland have been reviewed earlier in this chapter. Those considerations specific to craniopharyngioma are discussed here.

Because tumor resection may cause or exacerbate endocrine deficiencies, the management of these problems must begin preoperatively and continue through the postoperative period. Stress doses of hydroxycorticosteroids (e.g., hydrocortisone, 100 mg per $m^2$ intravenously followed by 25 mg per $m^2$ every 6 hours) are administered before, during, and immediately after the surgical procedure, often in addition to dexamethasone, which is used to reduce peritumoral edema. Doses then are tapered but, if postoperative endocrine testing demonstrates a need for long-term steroid hormone replacement, then hydrocortisone is continued at maintenance levels.

Hydrocephalus in patients with craniopharyngiomas generally resolves after the tumor has been resected. If the hydrocephalus is severe, a ventriculostomy can be inserted before the tumor resection is begun and can be removed within several days of surgery if the operative procedure opens the CSF pathways. If the hydrocephalus persists, a ventriculoperitoneal shunt is inserted.

The extent of surgical removal to be attempted is a matter of intense debate: Some authors strongly recommend radical surgery in all cases, [664,665] whereas others suggest partial resection followed by local irradiation. [206] The debate centers around the prognostic advantage gained by complete resection balanced by the morbidity associated with such a procedure. With the use of microsurgical techniques, a radiologically complete resection can be achieved in 60% to 90% of children.[660,664,665,666,667,668 and 669] Under these circumstances, the likelihood of long-term PFS is 80% to 90%. However, significant neurologic morbidity, memory and cognitive dysfunction, and appetite and neurobehavioral disturbances are encountered in 10% to 30% of patients, mortality ranges from 0% to 5%, and panhypopituitarism develops in 80% to 90% of patients. [656,659,664,665,670] The operative morbidity is lower in children operated on by neurosurgeons who perform such procedures frequently.[206] Although morbidity may also be lessened somewhat by more limited resections, the likelihood of long-term disease control is substantially lower than with complete resection. Without radiotherapy, the vast majority of subtotally resected tumors progresses within 2 to 5 years. [671,672] The recurrence rate is diminished significantly with the use of postoperative external irradiation, [206,673] although some studies indicate that the results, in terms of PFS, remain inferior to those achieved with total resection.[671] Although repeat microsurgical resection is feasible in patients with recurrent tumor, morbidity and mortality may be substantially higher than at the primary operation.[664] The primary cause of death in these patients is either recurrent tumor or chronic neuroendocrine problems. [674]

Craniopharyngiomas are extra-axial tumors, tenaciously attached to surrounding structures, such as the optic chiasm, hypothalamus, and vessels of the circle of Willis. These characteristics impose practical limits on a surgeon's attempt at tumor resection. Regardless of the degree of surgical resection intended at the outset, the usual cases are approached via a subfrontal or trans-sylvian exposure, working between the optic nerves, carotid arteries, and third nerves, or through the lamina terminalis. However, other approaches may be used, depending on tumor extent and location. A growing trend is to tailor the treatment strategy based on tumor size and composition. Tumors with a sizable solid component (greater than 3 cm) are treated microsurgically, generally via a transcranial approach; selected lesions that arise within and expand the sella turcica may be amenable to transsphenoidal resection. [675] Thin-walled cystic lesions can be treated using intracavitary techniques; if small residual solid components of the tumor subsequently enlarge, stereotactic radiosurgery or microsurgical techniques can be used. The management of small, solid tumors (less than 3 cm in diameter) remains particularly controversial, with most groups favoring microsurgical resection and others employing stereotactic techniques. For example, tumors located primarily in the sella can be removed transsphenoidally, [675] and large cystic tumors extending to the roof of the third ventricle can be approached through the corpus callosum.

***Radiotherapy***

Radiotherapy can decrease the recurrence rate and enhance the survival of patients with incomplete tumor resections ( Table 27-12). The addition of radiotherapy after subtotal tumor resection may result in a 50% to 80% disease-free survival. Even with minimal surgery, consisting of biopsy and cyst drainage, the survival of patients with both newly diagnosed and recurrent disease is prolonged by irradiation. Recommended doses of external beam irradiation are 50 to 55 Gy to local fields only.

| Treatment | 5 yr (%) | 10 yr (%) | References |
|---|---|---|---|
| Total resection | 58–100 | 24–100 | 662–665, 668, 673, 683, 684 |
| Subtotal resection | 37–71 | 31–52 | 139, 668, 671, 673, 683, 685 |
| Subtotal resection and irradiation | 69–95 | 62–84 | 662, 663, 668, 671, 673, 683, 685, 686 |

**TABLE 27-12. ACTUARIAL SURVIVAL RATES IN CRANIOPHARYNGIOMA WITH SURGERY AND RADIOTHERAPY**

Although a controlled trial has not been done, multiple comparisons strongly suggest that patients treated with incomplete tumor resection followed by irradiation have less neuroendocrine dysfunction and fewer serious sensory, motor, and visual deficits than do those who have undergone aggressive attempts at complete tumor resection. These patients may also have an improved level of function and better quality of life than patients treated with radical surgery alone. [656,670,676] Additionally, neuropsychological function is reportedly better preserved in the combined-therapy group despite the known detrimental effect of irradiation. Modern conformal radiation techniques appear to lower the morbidity also when compared to conventional techniques of irradiation.

Increasing interest has been generated in recent years in the use of stereotactic approaches to treat selected craniopharyngiomas. Predominantly cystic lesions, which account for 20% to 30% of tumors, may be treated with intracystic instillation of yttrium 90 or phosphorus 32 to deliver a cyst wall radiation dose of approximately 20,000 cGy.[677,678,679 and 680] Because of the limited tissue penetration of the radiation from these b-emitting isotopes, radiation exposure to the adjacent hypothalamus and optic nerves is minimized. Involution of the cyst is achieved in more than 80% of patients [678,679 and 680]; morbidity and mortality are lower than with microsurgical resection; and the recurrence rate is comparable to that achieved with attempted total microsurgical removal. [679,680] After intracavitary therapy, small, residual solid-tumor components can be treated with stereotactic radiosurgery. [677,680] This modality has also been used to treat residual or recurrent lesions after an initial microsurgical resection.

***Chemotherapy***

Chemotherapy has no established role in the treatment of craniopharyngiomas. An anecdotal response to a vincristine, BCNU, and procarbazine combination has been described in one patient.[681] Intracystic administration of bleomycin has also led to response and significant second remissions in some patients with recurrent disease,[682] but concerns have been raised regarding local toxicity.

## CHOROID PLEXUS NEOPLASMS

### Demography

Choroid plexus neoplasms constitute between 1% and 4% of brain tumors in children. Seventy percent of these occur during the first 2 years of life; the median age at diagnosis ranges from 10 to 32 months in recent series.[201,202 and 203,687,688,689,690 and 691] CPPs, benign tumors treated only surgically, outnumber the malignant counterpart, CPC, by nearly four to one.[201,687] Tumors arise from the lateral ventricles approximately 75% of the time, from the fourth ventricle and cerebellopontine angle in 15% of cases, and from the third ventricle in 10% of affected children. [688]

### Pathology and Patterns of Spread

Choroid plexus neoplasms generally arise as functioning intraventricular papillomas capable of secreting CSF. Grossly, CPPs resemble coral, fronds of tumor attached to a pedicle float in the CSF. Microscopically, these tumors are similar to normal choroid plexus and have cuboidal or columnar epithelium and a well-preserved epithelial-stromal border overlying fibrovascular septa ( Fig. 27-26). Their neoplastic nature is reflected in the heaping and redundancy of the epithelial component. These tumors tend to be slow-growing and, because of their intraventricular location, often reach a size of 60 to 70 g before they are diagnosed. Fewer than 5% are bilateral.

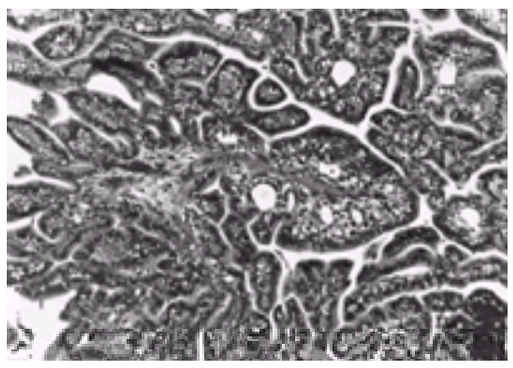


**FIGURE 27-26.** Choroid plexus papilloma. The photograph illustrates the luxurious papillary structures composed of a loose fibrovascular core covered by a single layer of cuboidal-columnar epithelium. (Hematoxylin and eosin, ×250.)

The CPC, a more aggressive and anaplastic tumor, accounts for up to 40% of choroid plexus neoplasms.[688,690,692] This tumor has lost the well-differentiated papillary structure and the epithelial-stromal border of the CPP (Fig. 27-27). It is a hypercellular tumor with pleomorphic cells, frequent mitoses, and foci of necrosis.[693] Both papillomas and carcinomas are capable of leptomeningeal dissemination. In CPPs, the clinical behavior and histology of the isolated and frequently noted deposits are benign, and symptoms are uncommon. Conversely, diffuse and aggressive leptomeningeal spread occurs in CPCs.

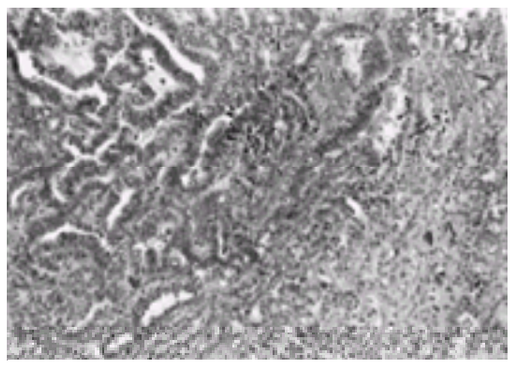


**FIGURE 27-27.** Choroid plexus carcinoma. The papillary character, which is partially retained in tissues on the left side of the field, has been lost in the portion of the tumor on the right side. Note pseudostratified epithelium forming irregular glandular structures on the left and diffuse epithelial growth on the right. (Hematoxylin and eosin, ×250.)

### Prognostic Considerations

Tumor histology and degree of resection are the primary prognostic factors for choroid plexus neoplasms. The long-term recurrence-free survival after complete resection of CPP approaches 100%.[688,694,695 and 696] Even less-than-complete resection is associated with long periods of PFS. The outcome is less favorable in patients with CPC,[202,203,697,698] because these lesions invade the brain parenchyma and are extremely vascular, making complete resection difficult. In addition, these tumors often disseminate within the CSF. Nevertheless, in reviews of experience with CPC, complete resection of disease appears to be the single variable that most affects long-term survival.[201,202 and 203,689,690] In fact, GTR of CPC may be curative by itself in a proportion of children.[692]

### Treatment

#### *Surgery*

Surgical excision is the primary mode of therapy for both CPP and CPC. These lesions most commonly arise in the trigone; tumors in this location usually are approached through a posterior temporoparietal craniotomy, through a cortical sulcus. Tumors of the anterior third ventricle and the body and frontal horn of the lateral ventricle may be approached transcallosally or transcortically through the middle frontal gyrus. Fourth ventricular tumors are approached by the suboccipital route. Intraventricular tumors outside the posterior fossa may be more easily removed if the ventricles are large; for this reason, preoperative shunts usually are not inserted in patients who are otherwise clinically stable. Contemporary surgical morbidity and mortality rates are less than 20% and 5%, respectively. Complete resections are possible in approximately 80% of patients.[688] Even after complete tumor removal, persistent hydrocephalus requiring a CSF shunt is present in up to 60% of patients.[699] If the postoperative MRI scan demonstrates residual tumor, reoperation is indicated. Appreciation for the importance of complete surgical resection has provided an impetus for efforts to perform second-look surgery in those children whose incompletely resected tumors persist after a trial of neoadjuvant chemotherapy.

#### *Radiotherapy*

Postoperative radiotherapy frequently is used to treat CPC, especially if the resection is incomplete or if there is evidence of leptomeningeal dissemination of disease. Although the survival times of some irradiated patients may be marginally better than those of nonirradiated patients, such results are not entirely separable from results with surgery alone. No randomized trial has yet tested the benefit of radiotherapy.

#### *Chemotherapy*

Surgery alone is usually sufficient for cure of CPP; chemotherapy has no role in the treatment of this tumor. As with other uncommon malignant pediatric CNS tumors, however, the role of chemotherapy is difficult to define in the treatment of CPC. Numerous reports of small numbers of patients collectively demonstrate that CPC can respond to different chemotherapeutic regimens at initial diagnosis or following relapse. The regimens given to the highest number of patients were based on platinum[201,687,688] or cyclophosphamide[692] or included multiple agents.[202] However, whereas most patients with CPC treated with GTR and chemotherapy appear to be long-term survivors, cure has been achieved with GTR alone. Furthermore, the majority of children whose tumors are less than completely resected and who also are treated with chemotherapy ultimately die of their disease.[201,202,690] Anecdotal reports, however, suggest that chemotherapy may render CPC less vascular and infiltrative and potentially amenable to complete removal, even if the initial attempt was unsuccessful.[202,687,697] Thus, chemotherapy may potentially contribute to higher chances of survival. Because the number of children with choroid plexus neoplasms is so low, international collaborative clinical trials will be needed to test hypotheses related to outcome of therapy.

## INTRAMEDULLARY SPINAL CORD TUMORS

### Demography

Intrinsic tumors of the spinal cord make up 1% to 10% of pediatric CNS tumors.[700,701,702,703 and 704] These tumors occur throughout childhood, and the median age at diagnosis is 10 years. Male patients are slightly more commonly affected than female patients, in a 1.3:1.0 ratio. Patients with neurofibromatosis appear to have a higher incidence of spinal cord tumors, as they do with other astrocytic neoplasms.

### Pathology and Patterns of Spread

In children, up to 70% of intramedullary tumors are astrocytomas. The remaining 30% are ependymomas (10%), other glial neoplasms such as oligodendroglioma and gangliogliomas (10%), and malignant gliomas (10%). Histologically, these tumors are indistinguishable from their intracranial counterparts. Large cysts, both within the tumor and at the superior and inferior margins, are common. In as many as 60% of cases, extensive involvement by tumor and cysts may be present. Slow and contiguous extension across several vertebral segments, with compression and effacement of normal tissues, is the usual mode of growth. [705,706 and 707] Leptomeningeal dissemination has been reported in as many as 58% of patients with high-grade tumors, but it is uncommon in patients with low-grade tumors. [707,708] The presence of multiple discrete tumors is associated with neurofibromatosis. Tumor location in the spinal cord appears to be random, with the incidence in each anatomic region (cervical, thoracic, lumbar) roughly proportional to the length of that region. The only exception is the myxopapillary ependymoma, which has a predilection for the conus medullaris and filum terminale.

### Prognostic Considerations

Spinal cord tumors in children are rare occurrences. The low number of patients and relative lack of inclusion in clinical trials with prescribed treatment approaches make the identification of published prognostic factors difficult. Bouffet et al. [704] reviewed the experience of 13 French treatment centers with spinal cord astrocytoma and found high-grade histology and short duration of presenting symptoms to be associated with poorer survival. [704] From review of other series, low-grade lesions are compatible with long-term survival, whereas this appears not to be the case with high-grade lesions. Although investigators have suggested that complete tumor removal may be associated with longer survival and less frequent local recurrences, the degree of resection has not been associated with outcome in several studies; however, such a determination is problematic in view of the small sizes of the study cohorts. [707,709,710 and 711] Ependymomas may be an exception to this generalization, in that patients who undergo total tumor resection have fewer recurrences than do those who undergo incomplete tumor resections. [712]

### Treatment

#### *Surgery*

Complete surgical resection is difficult for astrocytoma because a distinct tumor-cord interface often is absent, but extensive subtotal resections may be performed in most instances. Ependymomas are associated with a clearer cleavage plane and can usually be resected completely. Intramedullary tumors usually are approached by an osteoplastic laminectomy, removing as a single unit all laminae covering the solid portion of the tumor. Replacement of the lamina after surgery not only helps to protect the spinal cord but may also diminish the risk of subsequent spinal deformity. Operative mortality is low; morbidity and the amount of neurologic recovery are proportional to the severity of preoperative dysfunction and to the definition of the tumor-cord interface. In one report of 69 patients undergoing operations for intramedullary tumors, at a mean follow-up of 54 months, 17% were better than they had been preoperatively, 56% were unchanged, and 31% were worse. [713] Postoperative orthopedic follow-up and monitoring for spinal deformity are important. In 25% to 40% of children, the development or progression of such deformity occurs within a mean of 3 years.

#### *Radiotherapy*

No controlled trial of radiotherapy has been conducted in patients with intramedullary tumors, and evidence for its utility is inferred from the treatment of similar tumors in other CNS locations. As with low-grade lesions in the cerebrum, irradiation may be unnecessary for incompletely resected low-grade tumors. Evidence also suggests that radiotherapy may be unnecessary for completely resected ependymomas. Although radiation doses of 45 to 50 Gy have been used in patients with high-grade astrocytomas and in patients who have had incomplete resections of both astrocytomas and ependymomas, the effectiveness of this therapy has not been unequivocally documented.[707,709,711,714] These doses are lower than those usually given for gliomas and ependymal tumors in other locations because of the radiation tolerance of the spinal cord. The overall survival rates for LGAs with various degrees of resection and postoperative radiotherapy are 66% to 70% at 5 years, 55% to 73% at 10 years, and 67% at 20 years.[706,711,714] Even so, local recurrence rates have been as high as 33% to 86%. [707,714] Patients with anaplastic or high-grade tumors generally die of their disease within several months of diagnosis. For patients with ependymomas, survival rates of 50% to 100% at 5 years and 50% to 70% at 10 years are reported, and local recurrences are similarly high in patients with subtotally resected tumors. [705,714]

#### *Chemotherapy*

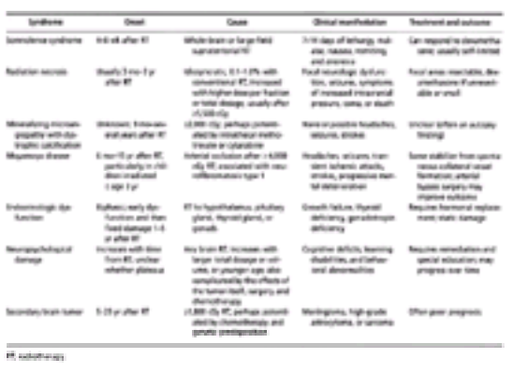
Tumors of the spinal cord have been treated as have their histologic counterparts in other parts of the brain. Chemotherapy has been employed for high-grade lesions at diagnosis, recurrent low-grade lesions, and in very young children in whom the avoidance of radical surgery or radiotherapy has been desired. [700,702,715] The largest of these series involved 13 children with high-grade astrocytoma of the spine who were treated with “8-in-1” chemotherapy along with radiotherapy, postoperatively. The response to pre-radiation chemotherapy was not measurable in three patients, was complete in one patient, partial in two, stable in four, mixed in two, and progressive in one. After completion of therapy, with the time of median follow-up not stated, 2 of the 13 patients were alive without disease at the report, and 5 were alive with disease. Five-year PFS and survival rates were 46% and 54%, respectively. [702]

Until biologic factors or other clinical trial results indicate otherwise, it seems rational to use chemotherapy for tumors of the spinal cord only as would be used for tumors of like histologies in other areas of the brain.

## SEQUELAE OF TREATMENT

Mortality rates for children with brain tumors have declined less rapidly than those of other cancers. [716] Nevertheless, the 5-year overall survival for children with brain tumors, aside from the favorable, LGAs, has improved to 60%.[5] Even when therapy is successful, these patients, unlike other long-term cancer survivors, often never overcome the symptoms and signs manifest at their tumor's clinical presentation. As a result, brain tumor survivors suffer physical, cognitive, neurologic, endocrinologic, and other deficits as sequelae of both their cancer and its therapy. These patients function at lower intellectual, social, and physical levels than their peers, leading to significant handicaps and diminished quality of life. The eventual magnitude of these problems may be greatest in patients who are the youngest at diagnosis (see Chapter 13, Chapter 15, and Chapter 49).

The acute and late effects among pediatric brain tumor patients arise from several sources. Before diagnosis, the tumor mass distorts and even destroys normal brain tissue and increases ICP, which sometimes is associated with hydrocephalus. Surgical trauma, postoperative meningitis, shunt infection, or repeat surgery can also cause some degree of irreversible neurologic damage.[717,718] Likewise, chemotherapy may be capable of producing encephalopathy. [719] Radiotherapy has been implicated as the chief cause of many adverse sequelae, which are listed in Table 27-13. Early on, irradiation may cause transient vasogenic edema, which exacerbates neurologic abnormalities and may require dexamethasone to alleviate symptoms. Other consequences are not apparent for years.



RT, radiotherapy

**TABLE 27-13. SYNDROMES OF POSTRADIATION DAMAGE WITH CHILDHOOD BRAIN TUMORS**

Cognitive impairment is among the most frequent and devastating problems of the child treated with radiotherapy. Although a drop in IQ is the most often cited effect of radiotherapy for a brain tumor, it is unclear whether IQ loss ultimately plateaus with age or continues longer. [720,721,722,723,724 and 725] This controversy is likely confounded by the lack of sensitivity of IQ testing to measure some neuro-cognitive effects. In fact, some children may maintain normal range scores because of intelligence and academic achievement before treatment. Scores within the average range of IQ do not necessarily indicate the child's level of adaptive functioning. More specific assessment of neuropsychological function usually will illustrate multiple areas of damage in information processing. Some studies have identified specific neuro-cognitive deficits in attention, memory, coordination, fine motor speed, visual motor processing, mathematics, and spatial relations. [721,725,726 and 727]

Extent of damage from irradiation is a complex function of the volume and dose, tumor location, and age at treatment. Even though IQ is an imprecise descriptor, reports agree that most young children receiving brain irradiation have a moderate to severe IQ drop, more striking with diminishing age of the child. [340,727,728] In one series of 29 survivors of medulloblastoma diagnosed at a median age of 2.5 years and treated with chemotherapy and then craniospinal irradiation at time of disease progression or completion of chemotherapy, the children had severe intellectual deficits. [729] IQ was 62 at a median follow-up of 5 years, as compared with a baseline of 88 at diagnosis. Although the loss of roughly 4 or more IQ points per year is startling, more alarming is that the cognitive losses had yet to plateau. Even among older children undergoing whole-brain irradiation, a linear decline in IQ is apparent, dependent on radiotherapy dose and age at treatment. [730] In one follow-up assessment of children with noncortical brain tumors receiving 24 to 36 Gy craniospinal radiotherapy, all patients younger than 7 years at diagnosis were receiving special education, and half of those patients older than 7 years were receiving supplemental educational services. [720]

The contribution of the volume of irradiation to cognitive deficits is perhaps more clear. Whole-brain irradiation appears to be linked to the greatest IQ decline and is more detrimental as a function of lower age of the child. [339,340,723,731] Supratentorial irradiation, which exposes the frontal and temporal lobes, hippocampus, and limbic structures, also leads to a significant IQ decrease. [732] Radiation fields limited to the posterior fossa appear to be associated with less intellectual damage, although IQ still drops. [728,731,732 and 733] When medulloblastoma patients who received 25 to 35 Gy craniospinal irradiation along with a boost to the posterior fossa totaling 45 to 55 Gy were compared to ependymoma patients receiving only the same posterior fossa doses, the differences were striking. [724] At 5- and 10-year follow-up, results remained stable for children with ependymoma, approximately 60% having an IQ in excess of 90. In contrast, medulloblastoma patients had progressive deterioration, 20% having an IQ of greater than 90 at 5 years after therapy and only 10% having this IQ at the 10-year follow-up.

The dosage of irradiation also clearly influences the development of cognitive deficits, with more severe sequelae occurring at higher doses. Patients who receive a dose of 36 Gy to the whole brain are estimated to score 8 points lower on IQ testing than those receiving 24-Gy doses, although both groups are subnormal on average. [339,730] Although the exact relationship of dosage to detrimental effect is not well defined, variable intellectual deficits and CT abnormalities of the brain occur regularly in young children with leukemia who are given doses of 24 Gy to the whole brain, and such abnormalities have been variably demonstrated even with lower whole-brain doses of 18 Gy. [345,722]

As another consequence of radiotherapy to whole brain, hypothalamic-pituitary region, or spine, growth failure occurs commonly among brain tumor patients. Irradiation along the spinal axis retards the growth of the vertebral column and spinal cord, leading to a child with a short trunk and disproportionately longer extremities. [719] Spinal irradiation alone has been associated with a decrease in eventual height of 9, 7, and 5.5 cm when administered at ages 1, 5, and 10 years, respectively. [734] A larger contribution to decreased stature stems from impaired GH secretion. After irradiation that includes the hypothalamic-pituitary axis, GH deficiency can be detected as early as 3 months after treatment and may become an almost universal finding in long-term survivors. [735] Abnormal responses to provocative GH testing and diminished growth velocity may become apparent after application of 29 Gy or less to the hypothalamic-pituitary region or 18 Gy to the craniospinal axis. [345,736] The use of concomitant chemotherapy may increase further the severity of growth retardation. [737] The effect of precocious puberty prematurely fusing bony epiphyses also can contribute to short stature.

Hormone replacement therapy should be considered in patients whose growth velocity has declined and who do not respond to provocative GH testing. The decision regarding whether and when to initiate replacement remains emotional and controversial for parents and physicians. Although the hormone is mitogenic, it does not appear to produce any increased risk for tumor recurrence. [738,739] Regardless, even when used, GH does not appear to correct fully the loss of stature caused by radiotherapy.

Other neuroendocrine deficits may also be seen. Primary, or less commonly secondary or tertiary, hypothyroidism may occur in more than half of patients as a result of irradiation to the thyroid gland or the hypothalamic-pituitary axis, respectively. [719,740] Puberty may occur prematurely or at normal onset but only seldom is delayed. Abnormalities of gonadotropin or corticotropin secretion may be less common, although this has not been examined rigorously in brain tumor survivors. [740] Male patients appear to be less at risk than female patients for gonadal dysfunction secondary to spinal irradiation, but the synergistic effect from commonly used drugs such as cyclophosphamide and nitrosoureas, known to affect oogenesis and spermatogenesis, has not been estimated. [719] Diabetes insipidus or panhypopituitarism follows radiotherapy rarely but seldom improves when present at diagnosis of a craniopharyngioma or germ cell tumor.

High-frequency, sensorineural hearing loss is another complication that is frequent among brain tumor survivors, most commonly caused by repeated administration of cisplatin. The contribution of radiotherapy to hearing loss appears to be less severe but less well studied. [741] In a recent CCG trial for medulloblastoma with 23.4 Gy craniospinal irradiation and a boost to the posterior fossa totaling 55.8 Gy, followed by adjuvant lomustine, vincristine, and cisplatin, the cisplatin dosage was halved or suspended because of ototoxicity in nearly one-third of patients. [364] Highly conformal radiotherapy may reduce the risk of ototoxicity.

Management of all these sequelae often is suboptimal. Due to the evident acute and long-term needs of these patients, a multidisciplinary team best manages them. This team should include not only physicians and nurses but also an occupational therapist, physical therapist, child life worker, educational psychologist, and social worker. The team and family must remember that effects may not occur or become fully manifest for 1 or several years after completion of therapy. Consequently, yearly psychological evaluations should be performed for at least the first 5 years after therapy. Children who demonstrate intellectual impairment require further evaluation so that proper educational intervention can begin. Even years after cranial irradiation, educational intervention has been associated with improvements in spelling and reading, particularly when written feedback was provided to parents and schools. [722] An evaluation by an occupational therapist may also be indicated to assess fine motor and visual skills. For at least 5 or more years, annual thyroid function studies should be performed to monitor for the often subclinical manifestations of hypothyroidism. Remediation is important to growth, learning, and prevention of thyroid tumors from persistently elevated thyroid-stimulating hormone. Other endocrinologic evaluations should be obtained as appropriate. Longitudinal audiometry should be considered for at least several years.

Long-term management of the child with a brain tumor also includes surveillance for disease relapse. Surveillance of brain tumor patients, unlike patients with other childhood cancers, is indeed a chronic issue. Progression or recurrence of tumors such as ependymoma or the LGAs frequently does not occur until 3 to 5 or more years from diagnosis. For medulloblastoma, with a median time to relapse of 18 to 24 months, recurrence may occur 8 or more years after diagnosis. [742] The ideal surveillance modalities and schedule for all the diverse tumor pathologies is unclear. Despite fair agreement that serial clinical examination is important not only to detect signs suggestive of relapse but also to monitor for the already mentioned sequelae of treatment, controversy surrounds the timing and need for surveillance MRI. For malignant brain tumors, the CCG has recommended surveillance MRI every 3 months during the first year after diagnosis, every 4 months during the second year, biannually during years 3 and 4 from diagnosis and, finally, annual to biennial scanning until 12 years after diagnosis. [743] Other investigators have questioned whether neuroimaging surveillance affects outcome, as the salvage rate for relapsed tumors such as medulloblastoma is low. [744,745] Further data are forthcoming from the POG experience to help answer this question. Even less clear is the decision regarding when to stop routine MRI scanning, as secondary brain tumors, particularly meningiomas, gliomas, and sarcomas, have been reported 5 to 25 years after treatment of the original tumor. [719] Most recommendations today are based on retrospective analyses. Prospective evaluation of neuroimaging surveillance schedules appropriate to the heterogeneous CNS tumor pathologies is needed.

Although prevention of these deleterious sequelae is beginning to receive attention, judging the efficacy of current interventions is difficult. Attempts to decrease, delay, or avoid radiotherapy have been made with the use of adjuvant or pre-irradiation chemotherapeutic regimens, particularly among very young children. However, several factors other than radiotherapy can influence neuro-cognitive and neuroendocrine function, and chemotherapy itself may have deleterious effects on the developing nervous system. [717,718,728,733] Furthermore, prolonged chemotherapy with alkylators and etoposide among infants with brain tumors has been associated with an excess risk of second malignancies. [439] Other new strategies to mitigate damage have yet to be tested. Conformal techniques can better focus the radiation delivered, such as in the posterior fossa where, in the past, local field treatment still exposed the hypothalamus and hippocampus just beyond the posterior clinoid processes. The chemoprotectant amifostine has yet to be widely studied with cisplatin administration.

Because most patients with neuro-cognitive deficits do not have observable histopathologic changes, the location and pathogenesis of their problems are not well

established. Thus, understanding the ways in which radiation and chemotherapy alter axonal growth, dendritic arborization and pruning, synaptogenesis, and myelination, all of which occur in childhood and adolescence, is presumably critical to understanding the development of these problems and remains largely unknown. Evidence suggests that MRI is capable of demonstrating areas of treatment-related white-matter abnormalities not visible on CT that may correlate with the degree of clinical neurologic compromise, particularly in IQ, factual knowledge, and verbal and nonverbal thinking. [339] Prospective evaluation with new MRI techniques and other novel neuroimaging modalities may improve understanding of the location and dynamics of damage associated with various therapies.

Imprecise methods, small patient numbers, and limited funding for studying patients with CNS tumors have been barriers to our knowledge of late effects. Closer collaboration with schools for assessment and intervention might help. In the future, comprehensive batteries that take the entire child into account are needed. Given that executive function, memory, pragmatic language, and other cognitive functions change over time, such evaluations should steer assessment away from IQ and toward developmental neuropsychological models. More precise definitions of disability and quality of life are needed. Newly validated instruments that measure quality of life should be implemented. Assessments will also have to consider practical outcomes, such as the ability to hold a job, drive a car, manage finances, or live independently.[741] Interventional trials of medications that improve arousal, attention, and memory, such as modafinil, methylphenidate, or donepezil, are needed also.

## INFORMATION ON CLINICAL TRIALS

For individuals wanting more information about clinical trials for children with brain tumors, the following resources are suggested:

| | |
|---|---|
| American Brain Tumor Association | 800.886.2282 |
| Children's Oncology Group | 800.458.6223 |
| National Cancer Institute | 301.496.6641 |
| Pediatric Brain Tumor Consortium | 301.496.6641 |
| Pediatric Brain Tumor Foundation | 800.253.6530 |
| The Children's Cause | 301.562.2765 |

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# RETINOBLASTOMA

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## INTRODUCTION

Retinoblastoma is a malignant tumor of the embryonic neural retina and is the most common intraocular malignancy in children. Although usually not recognized at birth, retinoblastoma is congenital and affects predominantly young children. The tumor has a variable growth rate, can originate from single or multiple foci in one or both eyes, and in bilateral cases, may be manifest in one eye many months before it is evident in the other. Retinoblastoma is caused by a mutation in a gene that expresses a protein central to the control of the cell cycle and may occur sporadically or be inherited. Children with the hereditary type of retinoblastoma have a particular susceptibility to developing other malignant tumors. This disease serves as a model for understanding the genetics and heredity of childhood cancer.

The term *retinoblastoma* was first adopted by the American Ophthalmological Society in 1926.[1] The cellular origin of retinoblastoma had been a topic of debate since 1809 when the Scottish surgeon Wardrop first recognized, based only on gross pathological findings, that retinoblastoma is a discrete tumor arising from the retina.[2,3] After this publication, other pathologists including Robin and Langenbeck confirmed the observations at a microscopic level. Virchow, however, thought that the cell of origin was glial and named it *glioma of the retina*. In the late 1800s, the term *neuroepithelioma* was proposed by Flexner, and supported later by Wintersteiner, because they believed that the tumor originated from the neuroepithelium and that the typical rosettes that now bear their names were attempts to form photoreceptors. In the early 1900s, Verhoeff concluded that the tumor was derived from undifferentiated embryonic retinal cells called *retinoblasts* and proposed the term *retinoblastoma*.[1] Margo and colleagues proposed the term *retinocytoma* for the well-differentiated tumor that displays benign features.[4] For this same tumor Gallie and colleagues described the clinical features and proposed the term *retinoma*.[5] The histopathological, ultrastructural, immunohistochemical, and molecular characteristics of retinoblastoma support the concept that this tumor originates from a multipotent precursor cell. This cell could develop into almost any type of inner or outer retinal cell, including photoreceptors.[6,7,8,9,10,11,12,13,14,15,16,17,18,19,20] and [21]

## EPIDEMIOLOGY

The Third National Cancer Survey indicates an average incidence of 11 new cases of retinoblastoma per million population younger than 5 years, or 1 in 18,000 live births in the United States.[22] Although data from developing countries are less complete, oncologists in Central and South America, the Middle East, and India generally feel that the incidence may be greater in these regions. The estimated frequency of bilateral retinoblastoma ranges from 20% to 30%. Thus, in the United States, an estimated 200 children per year develop retinoblastoma; of these 200, at least 40 to 60 cases are bilateral. There are no racial or gender predilections.

Retinoblastoma is often present at birth and is almost entirely restricted to early childhood. Approximately 80% of cases are diagnosed before children reach the age of 3 to 4 years, with a median age at diagnosis of 2 years.[23] The discovery of retinoblastoma beyond age 6 years is rare. Bilateral disease is diagnosed earlier than unilateral disease. Sporadic bilateral retinoblastoma has been associated with advanced parental age.[24]

Multiple congenital anomalies associated with retinoblastoma have been reported in approximately 0.05% of U.S. patients with retinoblastoma.[25] The reported anomalies include congenital cardiovascular defects, cleft palate, infantile cortical hyperostosis, dentinogenesis imperfecta, familial congenital cataracts, and incontinentia pigmenti, or Bloch-Sulzberger syndrome (an X-linked inherited disease that is lethal in males but affects females with pigmentary retinopathy, corneal opacities, cataracts, nystagmus, blue sclerae, myopia, pseudoglioma, dental abnormalities, abnormal skin pigmentation, and mental deficiency).[23] An association with mental retardation has been suggested in children with the D-deletion syndrome; however, most patients with retinoblastoma have no intellectual impairment.

## GENETICS

The majority of retinoblastomas appear sporadically; however, an inherited form of the disease has been documented[26,27] and is transmitted with few exceptions as a typical mendelian autosomal dominant trait with high but incomplete penetrance. Of all cases, approximately 60% are nonhereditary and unilateral, 15% are hereditary and unilateral, and 25% are hereditary and bilateral.[28,29]

A “two-hit” model has been proposed to explain the observations that familial cases are generally multifocal and bilateral, whereas sporadic cases typically present with unilateral unifocal disease at a later age.[28,30] According to the model, as few as two stochastic mutational events are required for tumor initiation, the first of which

can be inherited through the germ line (in heritable cases) or can occur somatically in individual retinal cells (in nonheritable cases). The second event occurs somatically in either case and leads to tumor formation from each doubly defective retinal cell.

The presence of a microscopically visible deletion in one chromosome 13 homologue in constitutional cells of a small number of retinoblastoma patients was the first evidence that supported an inherited mechanism for retinoblastoma development. [31,32,33 and 34] Although the deletions varied between families, each deletion minimally encompassed chromosome 13q14.[35,36] This chromosomal locus contains the RB1 retinoblastoma gene. In the two-hit model, such deletions in the germline could act as the first hit and confer the risk of tumor formation as an autosomal dominant trait. The increasing resolution of cytogenetic technology and the development of DNA probes for loci in the immediate vicinity of the RB1 gene locus has allowed the detection of more subtle genomic rearrangements. These techniques can be used to identify people who carry nonpenetrant mutations in the retinoblastoma susceptibility locus. [37]

Patients without a gross chromosome 13 deletion but who have bilateral or familial retinoblastoma have submicroscopic mutations at the RB1 locus similar to mutations that have been found in the tumor cells of patients with nonhereditary retinoblastoma. The second step in tumorigenesis in both heritable and nonhereditary retinoblastoma involves somatic alteration of the normal allele at the RB1 locus in such a way that the mutant allele is unmasked. Thus, the first mutation in this process, although it may be inherited as an autosomal dominant trait in the child, is in fact a recessive defect in the individual retinal cell. Elimination of the chromosome containing the wild-type allele followed by reduplication of the remaining mutant chromosome may be one mechanism by which the affected RB1 locus becomes homozygous within the cell.[38,39,40 and 41] The potential tumor cell becomes recessive for the mutant allele.

Although the unmasking of predisposing mutations at the RB1 locus occurs in mechanistically similar ways in sporadic and heritable retinoblastoma, only the latter carries the initial mutation in each cell. Patients with heritable disease also seem to be at greatly increased risk for the development of second primary tumors, particularly osteogenic sarcoma.[42] This high propensity is genetically determined by the predisposing RB1 mutation. The notion of a pathogenetic association between these two rare tumor types was tested by determining the constitutional and osteosarcoma genotypes at restriction fragment length polymorphism loci on chromosome 13. The data indicated that osteosarcomas arising in patients with retinoblastoma had become homozygous specifically around the chromosomal region carrying the RB1 locus.[43] Furthermore, these same chromosomal mechanisms eliciting losses of constitutional heterozygosity were observed in sporadic osteosarcomas, suggesting a genetic similarity in pathogenetic causality.

These studies provided data useful for the molecular isolation of the RB1 gene. [44] The genomic organization of the approximately 200-kb locus was determined, and the expression of its 4.7-kb messenger RNA transcript in tumor and normal tissues was documented. Introduction of the wild-type gene into retinoblastoma and osteosarcoma cell lines using recombinant retroviral vector transfer resulted in a partial reversal of the tumorigenic phenotype. [45,46] Further characterization of the complete RB1 genomic sequence[47] allowed a rigorous cataloging of the different mutations affecting the gene in retinoblastoma tumors. Over 200 disease-causing mutations have been identified in the retinoblastoma genes of patients. [37,48,49,50,51,52,53,54,55,56 and 57]

The examination of sporadic cases of bilateral retinoblastoma showed that disease frequently arises subsequent to a new germline mutation in the paternal allele, followed by somatic alteration or loss of the maternally derived wild-type allele. [58,59] This finding suggests either that mutations in the RB1 locus occur more commonly during spermatogenesis or that the paternal chromosome in the early embryo is at a higher risk of mutation. Analyses of sporadic osteosarcomas also showed preferential mutation of the paternal allele. [60]

Investigations of RB1 gene alterations at both the DNA and the RNA level cumulatively reveal a strong correlative relationship between the lack of RB1 gene product and the appearance of retinoblastoma tumors. In addition to osteosarcomas, other tumor types contain mutations involving the retinoblastoma ene. Molecular analyses of small cell lung carcinomas have revealed RB1 structural abnormalities in approximately 15% of cases. [61] Loss of heterozygosity for chromosome 13 has been detected in approximately 25% of breast cancers and related breast cancer–derived cell lines. [62,63] However, a more detailed analysis of the effects of chromosome 13 mutations in tumors has been compiled and clearly shows that not all tumors are either a direct or an indirect result of loss of heterozygosity of the RB1 locus.[64] The cumulative data suggest that only subsets of tumors may share a common pathogenetic mechanism that results from unmasking mutations affecting the tumor-suppressing function of RB1.

RB1 messenger RNA is a 4.7-kb transcript in normal human and rat tissues including brain, kidney, ovary, spleen, liver, placenta, and retina. [65] The expressed protein contains 928 amino acids and has an estimated molecular mass of 110 kd. Although the number of different types of tumors that occur as a result of inherited mutations of the RB1 locus is small, the broad tissue expression and species conservation of this gene suggest a common and potentially important role in the growth or differentiation of many cell types.

The protein has been shown to be primarily localized in the cell nucleus. [66] Posttranslational phosphorylation of the RB protein in quiescent cells overrides growth suppression and allows cell division to take place. [67] The RB protein also has a role in the regulation of the cell cycle of actively dividing cells. The unphosphorylated RB protein (p110RB) has been shown to bind E2F1, a transcription factor and a cell cycle regulator during the $G_1$ stage of the cell cycle. The RB/E2F1 complex masks the E2F1 transactivation domain and inhibits surrounding enhancer elements, thereby causing transcription of E2F1-regulated genes to cease. The RB protein accomplishes this by physically associating with histone deacetylase 1. This recruitment of the deacetylase to the E2F1 regulating domain by Rb allows for deacetylation of histone, thereby modulating the local structure of the chromatin. [68,69] Phosphorylation of the RB protein at the $G_1$/S boundary results in the release of these transcriptional factors, allowing them to become positive transcriptional elements. Additional cell cycle–specific kinases become activated and facilitate the progression of the cells through $G_2$ and M. At the completion of the cell cycle, phosphatases dephosphorylate the RB protein, allowing the protein to again sequester E2F1 and form an inactive complex. Thus, positive and negative regulation of transcription and, therefore, cell proliferation are linked to the phosphorylation cycle of the RB protein. In tumors in which RB protein is mutated or absent, these intracellular transcriptional elements are dissociated and free to promote consistent and uncontrolled progression through the cell cycle. Such behavior results in unchecked cell proliferation consistent with a malignant phenotype.

The viral oncoproteins of polyomaviruses (SV40), adenoviruses (Ad-2 and Ad-5), and papillomaviruses (HPV-16) have also been shown to complex with the RB protein.[70,71 and 72] Because one function of these viral oncoproteins appears to be the creation of a cellular environment that is permissive for DNA synthesis, one of their modes of action may involve sequestration of the antiproliferative unphosphorylated RB protein. Releasing the cell from its negative regulation by RB might allow the cell to enter S phase and synthesize DNA. Taken together, the data support a model in which the unphosphorylated form of RB is the species active in growth suppression.

### Genetic Counseling

Approximately 40% of patients with retinoblastoma have the inherited form of the disease. Because the inherited form of retinoblastoma is transmitted as an autosomal dominant trait with high but incomplete penetrance, there is a 45% chance that a child of the patient will inherit the disease. In addition, although there is high penetrance of the retinoblastoma phenotype, the possibility exists that one of the patient's siblings could also develop retinoblastoma even if neither of the parents was affected by the disease due to germline mosaicism and low penetrant alleles (see Chapter 3).[73,74 and 75] All children with a family history of retinoblastoma should be screened shortly after birth by a qualified ophthalmologist to permit early detection of the disease and increase the chance of ocular and vision salvage. These increased familial risks support the need for expert genetic counseling.

To effectively counsel patients and families of retinoblastoma patients, the underlying cause of the disease must be determined. Patients who present with bilateral disease can be assumed to have a germline mutation in the RB1 gene. Patients with unilateral disease at presentation may also have an underlying germline mutation. If a mutation in the RB1 gene is detected in the tumor, somatic cells should also be screened. A mutation initially detected in the somatic cells is presumptive evidence of a mutation in the germline. Genetic testing for the presence of this specific mutation in siblings or offspring should be pursued. These children can then be aggressively surveyed for the presence of emerging tumors. If genetic testing is not pursued, then tumor surveillance is recommended for all siblings of the affected patient. Current recommendations suggest examination at birth and every 4 months until age 4 years. For children with unilateral disease, genetic screening for RB mutations can now be offered to families at the time of enucleation. The testing requires a sample of tumor and peripheral blood from the patient[37] or, for patients with bilateral disease, a blood sample can be analyzed directly.

## CLINICAL PRESENTATION

Most cases of retinoblastoma in the United States are diagnosed while the tumor remains intraocular without local invasion or distant metastases. In developing countries, however, the diagnosis is frequently made only after an enlarged eye or gross orbital extension is apparent. These patients more commonly present with

local invasion.

The signs and symptoms of an intraocular tumor depend on its size and position. The most common presenting sign is leukocoria of one or both eyes ( Fig. 28-1). Leukocoria, a lack of the normal red reflex of the eye, is manifest when the tumor is large or has caused a total retinal detachment leading to a retrolental mass that is visible through the pupil. If vitreous hemorrhage occurs due to bleeding of the retinoblastoma vessels, the pupil may appear to have a dark reflex instead of the white reflex typically seen in retinoblastoma ( Fig. 28-2).[18,76,77] The second most common presenting sign is strabismus. Loss of central vision from a tumor in the macula may result in a disruption of the fusional reflex and cause the affected eye to drift.

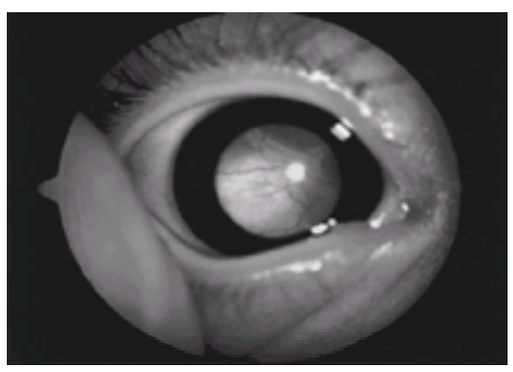

**FIGURE 28-1.** Photograph of the eye of a patient with retinoblastoma who presented with leukocoria.

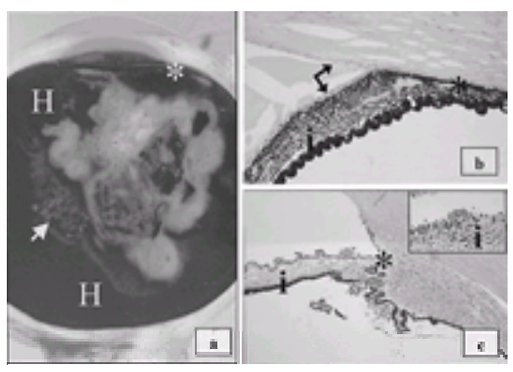


**FIGURE 28-2. A:** Gross photograph of an eye with retinoblastoma (white membranous tissue at center of the eye) with subretinal tumor seeds ( *arrow*) and vitreous and subretinal hemorrhages (H). Neovascularization of the anterior chamber and partial closure of the anterior angle ( *asterisk*) are also present. **B:** Histologic picture of rubeosis iridis showing neovascularization ( *arrows*) of the anterior portion of the iris (i) and focally on the endothelial surface of the cornea ( *arrows*). Contraction of the neovascular membrane produces closure of the anterior chamber angle ( *asterisk*). (Hematoxylin and eosin; original magnification 20×.) **C:** Histologic picture of the anterior segment of an eye with retinoblastoma seeds on the surface of the iris (i) with focal rosette formation ( *inset*). The anterior chamber angle (*asterisk*) is opened. (Hematoxylin and eosin; original magnification 20×.)

Other ophthalmic features accompany some cases of retinoblastoma and may indicate the necessity for immediate enucleation. Heterochromia (different color for each iris) may present as an initial sign of retinoblastoma secondary to iris neovascularization. The diagnosis of retinoblastoma should be excluded in children that present with this condition.[20] Rubeosis iridis (neovascularization of the surface of the iris) occurs in approximately 17% of patients with retinoblastoma and in more than 50% of patients with advanced retinoblastoma requiring enucleation ( Fig. 28-2).[20,76,79,80 and 81] Extensive necrosis of the tumor and liberated angiogenic factors may be responsible for this neovascularization of the iris.

Spontaneous bleeding from rubeosis iridis may also cause hyphema (blood in the anterior chamber), and the potential diagnosis of retinoblastoma should be investigated in a child presenting with spontaneous hyphema without history of trauma.[18,20,76,77] Glaucoma may be secondary to neovascularization of the anterior chamber angle or anterior synechia as a result of rubeosis iridis. Closed-angle glaucoma can also be secondary to mechanical obstruction of the anterior chamber angle by the iris and lens that has been pushed forward by a large intravitreal tumor. Most children with these presentations undergo enucleation.[18,76,77] Anterior chamber seedings from endophytic tumors or diffuse infiltrating tumors may produce pseudohypopyon (cells in the anterior chamber) ( Fig. 28-2). Intraocular tumors are not associated with pain unless secondary glaucoma or inflammation is present.

## DIAGNOSIS

Most commonly, a parent or relative of an affected child notes an abnormality of the eye that prompts physician evaluation. Current detection strategies involve a pediatrician looking for leukocoria using an ophthalmoscope. The gross appearance of a creamy pink to snow white mass projecting into the vitreous during the ophthalmoscopic examination (Fig. 28-1) may suggest retinoblastoma; however, associated findings of retinal detachment, vitreous hemorrhage, or opaque media often make inspection difficult. Pupillary dilation and examination with the patient under anesthesia are essential to evaluate the retina fully. Characteristically, the diagnosis is made by the ophthalmoscopic, radiographic, and ultrasonographic appearance, and pathologic confirmation is unnecessary. When the tumor is in an advanced stage, distinguishing vitreal seeding from multifocal tumors may be difficult; however, this distinction has important ramifications for the prognosis for the patient and for genetic counseling for the family. Earlier detection of the tumor would benefit the patient both by decreasing the chance of a child presenting with metastatic disease and by increasing the chance of being able to salvage the affected eye. A suggestion has been made to include dilation of the pupil before examination at the first well-child visit. An additional benefit of screening would be the earlier detection and treatment of congenital and infantile cataracts. Whether routine screening would be practical is controversial because diseases such as retinoblastoma and congenital cataracts are rare (congenital cataracts affect approximately 1 in 2,000 live births) and because pediatricians may not be adequately trained to recognize these conditions.

Ultrasonography and computed tomography (CT) (Fig. 28-3) of the orbit are the imaging studies most frequently used to confirm the diagnosis of retinoblastoma and to detect ectopic disease in the pineal gland.[82] Magnetic resonance imaging (MRI) of the orbit (Fig. 28-3) may be a more useful technique to detect tumor extension into the optic nerve and orbital coats.[83]

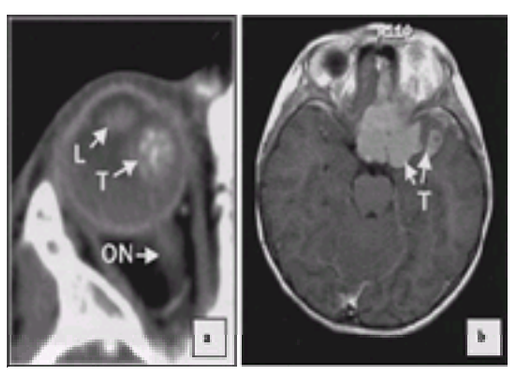


**FIGURE 28-3.** Diagnostic imaging of children with retinoblastoma. **A:** Computed tomography scan of the orbit. Axial view showing a partially calcified mass (T)

consistent with retinoblastoma, a normal lens (L), and normal optic nerve (ON). The globe is intact and shows no evidence of extraocular invasion by tumor. **B:** Magnetic resonance imaging of the orbits and brain. Contrast-enhanced coronal T1-weighted image showing parasellar and left middle fossa spread of retinoblastoma (T) with extension along the sylvian fissure. The eye on the left is normal. The orbit on the right contains a prosthesis.

The pretreatment evaluation must be individualized for each patient. In patients who present with small tumors, ultrasonography or CT scan of the orbits and careful examination under anesthesia may be all that is necessary to make the diagnosis. A more extensive metastatic workup is unnecessary in these patients unless there is a question of optic nerve extension or extensive choroidal invasion. A lumbar puncture to obtain cerebral spinal fluid cytology and CT or MRI of the brain to rule out brain metastases can be performed in those patients. Because of the rarity of distant metastases in patients with retinoblastoma, a bone marrow examination or bone scan is usually unwarranted unless the physician has suspicions of systemic involvement.

**Differential Diagnosis**

A number of benign conditions can clinically simulate retinoblastoma (pseudoretinoblastomas) and sometimes create considerable diagnostic difficulty for the ophthalmologist. Clinical definition is mandatory because the management of these entities differs considerably from the radical treatment of retinoblastoma.

Early reports of the frequency of enucleations performed for suspected retinoblastomas when an alternative final pathologic diagnosis is made varied from 30% to 16% according to the degree of oncologic experience of and the type of referrals received by the group reporting the series. [18,77,84,85] Most clinicians are now more familiar with pseudoretinoblastomas, and the frequency of erroneous enucleation is currently much lower. [20]

Other conditions that might be confused clinically also produce or simulate a mass in the vitreous or the retina. With the exception of medulloepithelioma, these lesions have in common a variety of histopathologic features distinct from retinoblastoma that create a difficult differential diagnosis for the pathologist.[79,86,87,88,89,90,91,92,93,94,95,96,97] and [98]

Approximately 60% of pseudoretinoblastomas include the differential diagnosis of three non-neoplastic entities: *Toxocara canis* endophthalmitis, persistent hyperplastic primary vitreous (PHPV), and Coats' disease.[18] All of these entities might present with retinal detachment and may have retrolenticular fibrosis. *T. canis* endophthalmitis is caused by the larvae of the nematode *T. canis* and presents almost always in children, although never at birth. Clinical history and serology are important for the diagnosis. Usually there are no signs of ocular inflammation, as the live larvae do not elicit an inflammatory response. Dead larvae elicit the formation of a localized eosinophilic abscess surrounding the microorganism. Condensed vitreous with gliosis and fibrosis may be present at the site of infection. Because these organisms are very small and degenerate, histological confirmation is very difficult.

PHPV is a congenital anomaly of the primary vitreous in which embryonal vessels do not regress and may pull the retina resulting in an anterior detachment. If the posterior capsule of the lens is ruptured by the traction of the vessels and fibrous membrane, a posterior subcapsular cataract forms. Some cases of PHPV are associated with a wide band to the optic nerve and with retinal dysplasia. There are rare cases reported in which PHPV has been associated with retinoblastoma.[99,100,101,102] and [103]

In contrast to toxocariasis and PHPV, Coats' disease lacks the fibrosis and vascularization of vitreous. Coats' disease is characterized by peripheral retinal vascular telangiectasis. These abnormal vessels leak and create an exudative retinal detachment rich in lipids with subretinal foamy macrophages and cholesterol clefts. Toxocariasis and PHPV simulate endophytic retinoblastoma, and Coats' disease mimics the exophytic type (see Pathology: Gross Features).[84,88,104,105,106,107] and [108] Ancillary imaging technology that includes ultrasound, CT scans, and MRI have greatly helped in the differential diagnosis of these lesions. [99,108,109,110,111,112] and [113]

**Use of Cytology in the Diagnosis of Retinoblastoma**

Retinoblastoma is the most frequent intraocular tumor of childhood in the United States, and in some other countries retinoblastoma is the most frequent intraocular tumor overall. However, retinoblastoma is one of the only human tumors that is radically treated without tissue biopsy confirmation. The clinical presentation and the ancillary radiologic and ultrasonic findings are typical for retinoblastoma in the majority of patients. Usually the correct diagnosis does not represent a diagnostic dilemma for the experienced pediatric/oncologic ophthalmologist. The resistance to biopsy confirmation of the tumor arises from the dramatic difference between survival for patients with contained intraocular tumors versus those with extraocular seeding of the tumor. The bias against biopsy is also aggravated by the reports of cases in which the tumor was misdiagnosed as “uveitis” or obscured by cataract, and patients developed orbital extensions of retinoblastoma after vitrectomy. [114]

The development of more refined techniques of fine-needle aspiration biopsy (FNAB) and the increased knowledge of the biological behavior of retinoblastoma has allowed some patients to benefit from pretreatment biopsy. [104,115,116,117,118,119,120,121] and [122] The FNAB technique is more difficult to perform; however, FNAB is safer for the patient because it prevents tumor seedings by avoiding the subconjunctival and scleral/orbital routes of entry. The 30-gauge needle passes through the peripheral cornea, anterior chamber, peripheral iris, lens zonules (avoiding puncture of the lens), and vitreous and penetrates the tumor ( Fig. 28-4). There have been no reports of extraocular tumor spread through the needle tract when FNAB has been used. FNAB is recommended only in selected cases in which the diagnosis is ambiguous and when adequate steps are taken to prevent extraocular seeding of tumor cells.

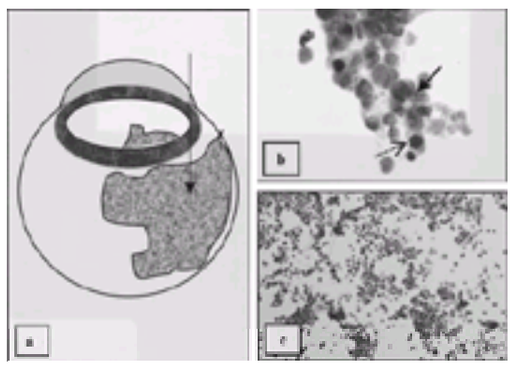

**FIGURE 28-4. A:** Drawing of an eye with retinoblastoma indicating the entrance of the needle ( *arrow*) through the peripheral cornea and peripheral iris, then between the ciliary body and the lens into the tumor. The technique avoids vascularized conjunctiva of the limbus and the orbit, sclera, and pars plana, preventing possible spreading of tumor cells via the needle tract. **B:** Cytologic preparation of a retinoblastoma showing cohesive groups of neoplastic cells with high nuclear to cytoplasmic ratio, increased mitotic activity (*dotted arrow*), and focal rosette formation (*solid arrow*). (Papanicolaou; original magnification 100×.) **C:** Cytologic preparation of cerebrospinal fluid in a patient with retinoblastoma metastatic to the brain. Notice the cohesive groups of neoplastic cells with high nuclear to cytoplasmic ratio. (Hematoxylin and eosin; original magnification 20×.)

The cytological findings are those of small- to medium-sized basophilic cells with scanty cytoplasm that tend to group together (rosettelike). Mitoses may be easy to find, and necrosis is frequently encountered ( Fig. 28-4). Similar features can be seen in cytologic cerebrospinal fluid specimens in children with intracranial metastases (Fig. 28-4). In one series of FNAB of pediatric intraocular tumors, the overall accuracy of FNAB was 95%, and the accuracy of cytologic interpretation was 100%. Therefore FNAB is a reliable and accurate diagnostic tool for the assessment of selected pediatric ophthalmic diseases in which the diagnosis is in question.[123,124]

## PATHOLOGY

## Gross Features

Primary retinoblastomas originate in the sensory retina and occupy the retina and vitreal cavity. Retinoblastoma is usually white-gray with a chalky appearance and a soft, friable consistency. Bright white speckles corresponding to calcifications present throughout the tumor. The gross features of retinoblastoma depend on the growth pattern of the tumor.[18,21,76] Some of these patterns correlate with clinical presentations and differences in biological behavior, especially as they relate to intraocular and extraocular types of tumor spread.

The endophytic growth pattern is represented by tumors arising from the retina and growing into the vitreal cavity ( Fig. 28-5). These tumors tend to entirely fill the cavity and produce floating tumor spheres called *vitreal seeds*. Tumor left untreated eventually invades the anterior portion of the eye, reaching the aqueous venous channels and the conjunctiva. From there, the tumor can permeate the lymphatic vessels and metastasize to regional lymph nodes. [18,21,125,126] and [127]

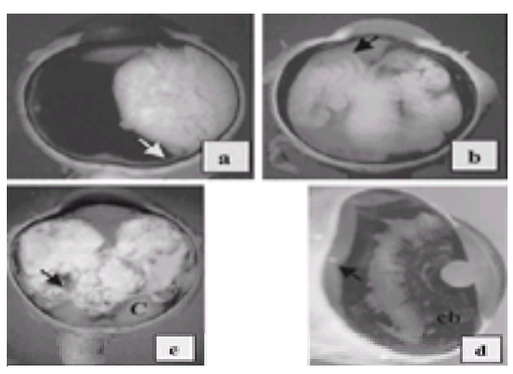


**FIGURE 28-5. A:** Gross photograph of an eye with a retinoblastoma showing an endophytic growth pattern. Notice that the tumor mass is growing from the retina (*arrow*) into the vitreal cavity. **B:** Gross photograph of an eye with a retinoblastoma showing an exophytic growth pattern. Notice that the tumor is growing from the retina (*arrow*) into the subretinal space with associated retinal detachment. **C:** Gross photograph of an eye with a retinoblastoma showing a mixed growth pattern, the most frequent type. Notice that the tumor grows both into the vitreous cavity and into the subretinal space with the retina ( *arrow*) entrapped in the middle. The tumor has massively invaded the choroid (C). **D:** Gross photograph of an eye with a diffuse retinoblastoma. Notice the absence of a well-formed mass; instead there are white seeds of tumor cells along the retina (*arrow*) and ciliary body (cb).

Exophytic tumors grow from the retina into the subretinal space and often cause serous detachments of the retina ( Fig. 28-5). These tumors may invade the choroid through Bruch's membrane.[18,21,128,129] and [130] Mixed endophytic and exophytic tumor growth is the most common pattern encountered (Fig. 28-5).[18,20,21]

Diffuse infiltrating retinoblastoma is the least common tumor growth pattern but is the most diagnostically challenging type because there is no predominant mass (Fig. 28-5). This presentation of retinoblastoma is seen in children with an average age of 6 years. The tumor cells grow throughout the retina while single cells and vitreal seeds invade the anterior portions of the retina, the ciliary body, and eventually the anterior chamber. Clinically this type of tumor resembles an inflammatory process with pseudohypopyon mimicking inflammatory cell accumulation (hypopyon) and vitreal seeds simulating the inflammatory cellular reaction seen in uveitis. Because this type of retinoblastoma resembles an inflammatory process, the diagnosis is often delayed until cytological examination of the aqueous humor or, in rare cases, of the vitreous. Almost all reported cases have a unilateral, sporadic presentation without family history. Although the diagnosis is difficult, children with diffuse infiltrating retinoblastoma have a good prognosis after enucleation. [101,127,131,132,133,134,135,136,137,138,139] and [140] Any child, regardless of age, who presents with signs of endophthalmitis should be considered to have diffuse infiltrating retinoblastoma until proved otherwise. [138]

Complete spontaneous tumor regression through unknown mechanisms occurs more commonly in retinoblastoma than in any other malignant tumor. In most of these cases complete occlusion of the central retinal artery is found; however, it is unknown whether this is a primary event or the result of tumor necrosis. [141,142] Severe inflammatory reaction with massive necrosis of the tumor followed by phthisis bulbi (complete atrophy of the eye) is the usual presentation.[141,142,143,144,145,146,147,148,149,150,151,152] and [153] If the eye is enucleated at the time of acute necrosis, the gross findings are those of massive tumor necrosis with edema of the conjunctiva.[141,154] If the eye is examined after complete atrophy has occurred, the findings are those of a small shrunken eye with mostly necrotic calcified tumor and a disorganized retina.

## Histologic Features

Microscopic examination of the affected eye displays one or more tumors with large areas of necrosis and multifocal calcifications replacing portions of the retina ( Fig. 28-6). The majority of the tumor is formed by small hyperchromatic cells with a high nuclear to cytoplasmic ratio. The tumor cells are mitotically active but frequently exhibit apoptosis (Fig. 28-6).[18,20,21,76,95,155,156,157,158] and [159] The viable cells surround blood vessels in a range of 90 to 110 mm forming a collarette (pseudorosettes) ( Fig. 28-6). Viability of the tumor cells depends on the intrinsic tumor blood supply. Areas of coagulative necrosis contain multiple foci of dystrophic calcification. Tumor cell necrosis liberates DNA from the nuclei of the cells. The released DNA forms deposits on the basement membranes of the vessels, the lens (capsule), the retina (internal limiting membrane), and the choroid (Bruch's membrane) ( Fig. 28-6).[160]

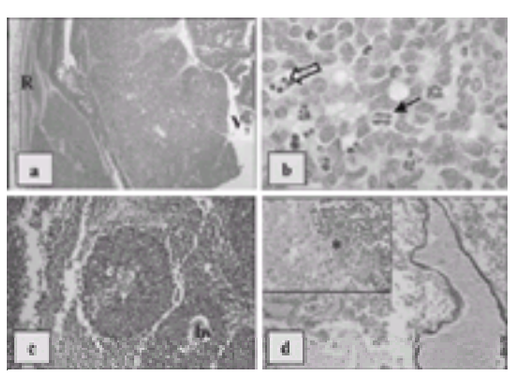


**FIGURE 28-6. A:** Histologic picture of a retinoblastoma growing from the retina (R) into the vitreous cavity (V). (Hematoxylin and eosin; original magnification 4×.) **B:** Histologic photograph showing poorly differentiated retinoblastoma cells with high nuclear to cytoplasmic ratio, increased mitotic activity ( *solid arrow*), and increased apoptosis (*open arrow*). (Hematoxylin and eosin; original magnification 100×.) **C:** Microphotograph showing viable tumor cells surrounding blood vessels (bv) with necrotic cells beyond a rim of approximately 110 µm. (Hematoxylin and eosin stain; original magnification 20×.) **D:** Microphotograph of retinoblastoma with extensive necrosis (*inset*) with foci of calcification (*asterisk*). Notice the large vessel darkly stained with hematoxylin (basophilic) secondary to DNA deposits in the vascular basement membrane as a result of extensive tumor cell necrosis that liberates nuclear DNA. (Hematoxylin and eosin; original magnification 10×.)

Some retinoblastomas show large areas of undifferentiated or poorly differentiated tumor ( Fig. 28-7); other retinoblastomas show a certain degree of differentiation represented by the formation of rosettes. Flexner-Wintersteiner rosettes are highly characteristic of retinoblastoma although they are also seen in pinealoblastomas and medulloepitheliomas. Flexner-Wintersteiner rosettes are lined by tall cuboidal cells that circumscribe an apical lumen. The apical ends attach to each other by terminal bars, and the cells may have apical cytoplasmic projections into the lumen of the rosette ( Fig. 28-7). Electron microscopy has demonstrated that these projections represent inner and outer segments of photoreceptors.[93,161,162] This and several other observations support the idea that retinoblastomas arise from

undifferentiated retinal cells that may differentiate into photoreceptors, [8,93,161,162,163,164,165,166 and 167] usually of the cone cell lineage.[6,7] Homer Wright rosettes are less common than Flexner-Wintersteiner rosettes, and they are found in a variety of neuroblastic tumors in addition to retinoblastoma. These rosettes do not surround a lumen but rather extend cytoplasmic processes that fill the center of the rosette. Homer Wright rosettes may be incomplete and admixed with well-formed Flexner-Wintersteiner rosettes (Fig. 28-7).

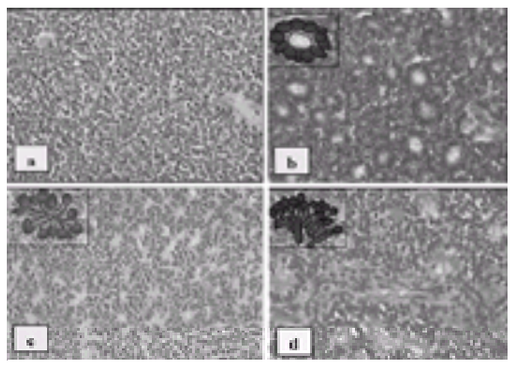

**FIGURE 28-7. A:** Microphotograph of a poorly differentiated retinoblastoma showing sheets of neoplastic cells without rosette formation. (Hematoxylin and eosin; original magnification 40×.) **B:** Microphotograph of a retinoblastoma showing Flexner-Wintersteiner rosette formation ( *inset*). Notice that these rosettes have a center partially filled by cytoplasmic prolongations with apical terminal bars. (Hematoxylin and eosin; original magnification 40×.) **C:** Microphotograph of a retinoblastoma showing Homer Wright rosette formation (*inset*). The lumen of the rosette is filled by cytoplasmic prolongations. (Hematoxylin and eosin; original magnification 40×.) **D:** Microphotograph of a well-differentiated retinoblastoma showing fleurettes ( *inset*). Fleurettes are groups of well-differentiated cells similar to photoreceptors, joined by cytoplasmic junctions and forming a figure similar to a bouquet of flowers. (Hematoxylin and eosin; original magnification 40×.)

Approximately 6% of tumors show benign photoreceptor differentiation into groups of cells with short cytoplasmic processes, abundant cytoplasm, and small round nuclei similar to photoreceptors. These groups of cells, which resemble a bouquet of flowers, are called *fleurettes*.[16,168,169] Neither significant mitotic activity nor necrosis is observed within the fleurettes (Fig. 28-7).[170,171 and 172]

### Retinocytoma

A benign counterpart of retinoblastoma called *retinocytoma* (also *retinoma*) that solely contains well-differentiated glial cells and fleurettes has recently been described. These benign tumors contain areas of abrupt calcification associated with retinal pigment epithelium proliferation ( Fig. 28-8). They exhibit specific features that allow experienced clinicians to follow the behavior of the tumor without radical treatment. [4,9,76,173,174] Singh and colleagues[173] reported on the ophthalmoscopic features of 24 tumors considered to be characteristic of retinocytoma, including the presence of a translucent retinal mass in 21 (88%), calcification in 15 (63%), and retinal pigment epithelial alteration in 13 (54%) of the tumors. A combination of all three features was observed in 8 (33%) of the 24 tumors. In 13 (54%) of the tumors, a zone of chorioretinal atrophy could be observed. Although the majority of these tumors behave as benign lesions, close follow-up is suggested because a few tumors have been reported to have undergone malignant transformation into retinoblastomas that eventually required enucleation. [168,173]

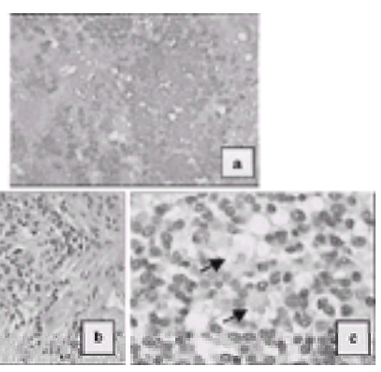

**FIGURE 28-8. A:** Histologic picture of abrupt cell calcification in a retinocytoma. (Hematoxylin and eosin; original magnification 40×.) **B:** Histologic picture of a well-differentiated area of a retinocytoma showing glial and neural differentiation ( *asterisk*). (Hematoxylin and eosin; original magnification 40×.) **C:** Histologic picture of a well-differentiated area of a retinocytoma showing fleurettes ( *arrows*). (Hematoxylin and eosin; original magnification 40×.)

In retinocytoma tumors that have undergone complete regression, either spontaneously or secondary to treatment, mummified calcified tumor cells and large areas of dystrophic calcification are observed. Exuberant reactive retinal pigment epithelial proliferation, ciliary epithelial cells, and glial cells with occasional ossification accompany this process.[76]

## METASTASIS AND RECURRENCE

If left untreated, retinoblastoma usually fills the eye and completely destroys the internal architecture of the globe. The most common route of spread is by invasion through the optic nerve. Once in the nerve, tumor spreads directly along the nerve fiber bundles toward the optic chiasm or infiltrates through the pia into the subarachnoid space. From the subarachnoid space, the retinoblastoma can involve the cerebrospinal fluid, the brain, and the spine. The second major route of spread is through massive involvement of the choroid into the orbit via either scleral canals (areas within the sclera in which ciliary vessels, nerves, and vortex veins enter or exit the eye) or by direct extension through the sclera.[175] Extraocular extension generally occurs within 6 months if intraocular tumors are left untreated.

Extraocular extension dramatically increases the chances of hematogenous and lymphatic spread. There are four routes for metastatic spread of retinoblastoma. [18,76]

1. Metastatic spread can occur by direct infiltration either through the optic nerve into the brain or through the choroid into the orbit soft tissues and bones.
2. Dispersion of the tumor cells through the subarachnoid space of the optic nerve into the opposite optic nerve or through the cerebrospinal fluid into the brain and spine can cause metastatic spread of the tumor. This can occur without detectable presence of retinoblastoma at the surgical margin of the optic nerve.
3. Metastasis can occur by hematogenous dissemination secondary to orbital and bone invasion or when lymphatic invasion reaches the lymph nodes. Widespread metastasis can present in lung, bone, and brain, among other sites.
4. Metastasis via lymphatic dissemination occurs in tumors that spread anteriorly into the conjunctiva and eyelids or extend into extraocular tissues. Lymphatic vessels and lymphoid tissue are absent in the orbit and intraocular tissues. In the ocular region, only conjunctiva and skin have lymphatic channels. Tumors must first reach these areas to permeate the lymphatic vessels and then spread into regional lymph nodes.

Histologically, retinoblastoma metastases appear less differentiated than intraocular tumors. Rosettes are rarely encountered, and fleurettes have never been described. When very well-differentiated extraocular tumors appear outside of the orbit, a differential diagnosis of a primary primitive neuroectodermal tumor must be considered.

## TRILATERAL RETINOBLASTOMA AND OTHER TUMORS

Primary retinoblastomas of the pineal and parasellar sites have been called *trilateral retinoblastoma* and usually present as single tumors. Trilateral retinoblastoma is

a well-recognized, although rare, syndrome.[176] The majority of the reported cases have involved patients with a family history of retinoblastoma, and the disease is usually fatal. These tumors may appear several years after successful treatment of intraocular retinoblastoma. They may be far more differentiated than the primary tumor and may contain numerous rosettes, fleurettes, and individual cells showing photoreceptor differentiation. The presentation of trilateral retinoblastoma contrasts with metastatic retinoblastoma because metastatic retinoblastoma presents as multiple, undifferentiated tumors within the first 2 years of initial treatment.

## STAGING

Different classifications have been introduced as guidelines for predicting prognosis for vision, globe salvage, and life. The Reese-Ellsworth classification ( Table 28-1)[177] relates to eyes treated by methods other than enucleation—specifically, radiotherapy. This classification, devised before the development of current ophthalmologic methodologies for diagnosis and treatment, has often been used to imply prognosis for life rather than for vision. Although the Reese-Ellsworth classification is not necessarily prognostic for outcomes using modern treatment modalities, it is still the classification used most often to compare therapeutic results. Other staging systems have been published which classify disease within or beyond the globe. [178] The American Joint Committee on Cancer has proposed a clinical and pathologic staging classification for retinoblastoma in which complete spontaneous regression of the tumor has not occurred. Using these criteria for cases of bilateral retinoblastoma, each eye is staged separately. Histologic verification of the disease in an enucleated eye is required, and any unconfirmed cases must be reported separately. The extent of retinal involvement is indicated as a percentage of the total retinal area. For the pathologic staging, all of the clinical and pathologic data from the resected specimen are to be used. A revised staging classification that would better predict clinical outcome using current therapeutic modalities is necessary.

| | | |
|---|---|---|
| Group I | | Very favorable |
| | a | Solitary tumor, <4 disk diameters in size, at or behind the equator |
| | b | Multiple tumors, none >4 disk diameters in size, all at or behind the equator |
| Group II | | Favorable |
| | a | Solitary tumor, 4–10 disk diameters in size, at or behind the equator |
| | b | Multiple tumors, 4–10 disk diameters in size, behind the equator |
| Group III | | Doubtful |
| | a | Any lesion anterior to the equator |
| | b | Solitary tumors >10 disk diameters behind the equator |
| Group IV | | Unfavorable |
| | a | Multiple tumors, some >10 disk diameters |
| | b | Any lesion extending anteriorly to the ora serrata |
| Group V | | Very unfavorable |
| | a | Massive tumors involving more than half the retina |
| | b | Vitreous seeding |

*Refers to chances of salvaging the affected eye and not systemic prognosis.

**TABLE 28-1. REESE-ELLSWORTH CLASSIFICATION FOR CONSERVATIVE TREATMENT OF RETINOBLASTOMA[a]**

## PROGNOSTIC FACTORS

Prognosis for vision in children with unilateral retinoblastoma is excellent for the uninvolved eye. The development of tumors in the contralateral eye after 3 years is very rare. A primary tumor with vitreous, subretinal, and retinal seeds can be mistaken as a multifocal primary tumor. Primary tumors arise from the sensory retina, whereas retinal seedings sit on top of the retina ( Fig. 28-9) or on the inner subretinal surface of the photoreceptors. The presence of multiple primary tumors or the emergence of tumors in both eyes (bilateral retinoblastoma) supports a diagnosis of inherited retinoblastoma. The prognosis for vision in bilateral retinoblastoma depends on the extent of tumor involvement and the effectiveness of treatment modalities. If the tumors are small and away from the fovea (central portion of the retina with best visual acuity), one may anticipate a good prognosis for vision after successful treatment. [18,21,76]

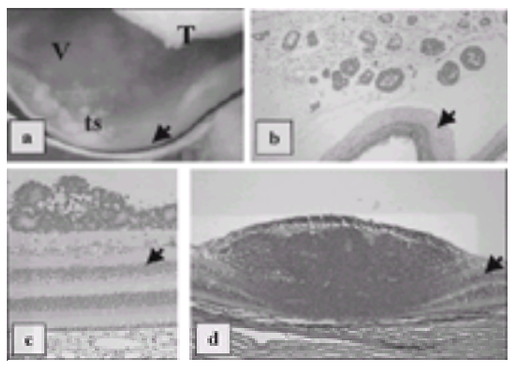


**FIGURE 28-9. A:** Gross photograph showing a primary retinoblastoma tumor (T) with tumor seeds (ts) on the inner retina ( *arrow*) and in the vitreous (Y). **B**: Microphotograph of an area of vitreous tumor seeds. Notice the hollow center of the retinoblastoma seeds. The retina ( *arrow*) is without tumor. (Hematoxylin and eosin; original magnification 10×.) **C:** Microphotograph of a retinoblastoma seed on the inner surface of the retina. Notice the rosette formation of the tumor and the intact architecture of the retina (*arrow*). (Hematoxylin and eosin; original magnification 40×.) **D:** Microphotograph of a retinoblastoma arising from the retina ( *arrow*). Notice the tumor replacing the normal architecture of the retina. (Hematoxylin and eosin; original magnification 20×.)

The survival rate from retinoblastoma has improved dramatically over the last century. One of the first retinoblastoma survival studies was reported in 1897 by Wintersteiner.[179] That 13% survival rate is in sharp contrast to the 90% overall 5-year survival reported by many centers today. [180,181,182 and 183] The main reasons for this improvement are the improved ability to detect retinoblastoma before the onset of metastatic disease and the development of alternative treatment strategies (see Therapeutic Options).

Metastatic disease is still associated with a poor prognosis. Most clinical findings are not useful in predicting the occurrence of metastasis in children with retinoblastoma, although histopathologic data provide a fair estimate of its risk. Multivariate statistical analysis has suggested the correlation of certain histopathologic findings and prognostic risk factors.[182,184,185,186,187 and 188] The most important prognostic indicators for the development of metastasis are the presence of tumor in the optic nerve posterior to the lamina cribrosa at the site of surgical transection and extrascleral extension of tumor into the orbit. [104,125,126,129,175,182,184,188,189,190,191,192,193,194 and 195] The extent of tumor invasion in the optic nerve correlates with prognosis ( Fig. 28-10). Superficial invasion of the optic disk is associated with a mortality rate of 10%, a rate similar to that seen when the optic nerve is not involved. The presence of tumor up to the lamina cribrosa is associated with a mortality rate of 29%. Invasion of tumor posterior to the lamina cribrosa is associated with a mortality rate of 42%, whereas the presence of tumor at the transected surgical margin is associated with a mortality of 80%.[125,175,182,184,188,196] The importance of obtaining a large portion of optic nerve at the time of enucleation is underscored by these results. Specific studies related to the length of the optic nerve stump alone show that patients with the optic nerve measuring less than 5 mm in the enucleated eye have a worse prognosis than those having stumps longer than 5 mm.[182,188,192,197,198]

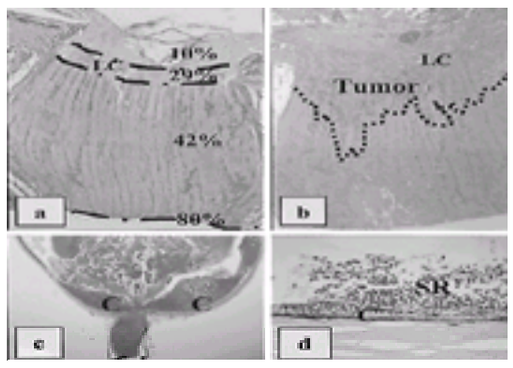


**FIGURE 28-10. A:** Microphotograph of a normal optic nerve showing the prognostic percentages of survival of patients with invasion of the optic nerve by anatomic portions of the nerve. Patients with tumors invading the pre–lamina cribrosa have a 10% mortality rate similar to that seen without invasion of the nerve. Invasion into the lamina cribrosa (LC; *between the two dotted lines*) carries a 29% mortality rate, and invasion beyond the lamina cribrosa carries a 42% mortality rate. Patients with tumors that are present at the surgical margin of resection (*single dotted line*) have an 80% mortality rate. (Myelin stain; original magnification 10×.) **B:** Microphotograph of an optic nerve showing tumor invasion beyond the lamina cribrosa (LC) but not at the surgical margin of resection. (Hematoxylin and eosin; original magnification 10×.) **C:** Microphotograph of the posterior pole and optic nerve of an eye with massive involvement of the choroid (C) and optic nerve by tumor. Notice that retinoblastoma tumor is present at the surgical margin of resection of the optic nerve ( *single dotted line*). (Hematoxylin and eosin; original magnification 4×.) **D:** Microphotograph of the subretinal space (SR) and choroid (C) of an eye with retinoblastoma with focal involvement of the subretinal space and minimal involvement of the choroid. (Hematoxylin and eosin; original magnification 20×.)

Massive, but not focal, invasion of the choroid by tumor increases the possibility for hematogenous spread, either through vascular permeation of choroidal vessels or, more frequently, by extension through the sclera into the orbital tissues ( Fig. 28-10).[125,182,187,189] MRI studies are helpful in evaluating the extent of involvement of choroid or optic nerve by tumor.[113]

Retinoblastomas that are poorly differentiated tend to behave more aggressively and are associated with a worse prognosis. Other factors associated with some risk for metastatic behavior, especially in conjunction with the major factors cited above, are tumor invasion into the anterior chamber and large tumor size with vitreous seeding, rubeosis iridis, and glaucoma.

## THERAPEUTIC OPTIONS

The management of retinoblastoma is complex. The diagnosis and treatment of patients with retinoblastoma involve a team approach requiring pediatric oncologists, ophthalmologists, and radiologists skilled in the treatment of patients with retinoblastoma. An important team role is also filled by child psychologists, social workers, nurses, and genetic counselors who can support families dealing with the difficulties of caring for a child who not only has cancer but also may lose an eye and vision. The goals of treatment are, most important, to save the child's life and, secondly, to salvage the eye or vision. Therapy is tailored to each individual case and is based on the overall situation, including threat of metastatic disease, risks for second cancers, systemic status, laterality of the disease, size and location of the tumor(s), and visual prognosis. There are several medical and surgical options for treatment of retinoblastoma, and the ocular oncologist should be thoroughly familiar with the indications, techniques, and expected results, as well as the associated systemic and visual problems of all treatment methods. [199] The currently available treatment methods for retinoblastoma include enucleation, external beam radiotherapy, plaque radiotherapy, laser photocoagulation, cryotherapy, thermotherapy, chemothermotherapy, intravenous chemoreduction, subconjunctival chemoreduction, systemic chemotherapy for possible metastatic disease, and orbital exenteration.

### Enucleation

Enucleation is still the treatment of choice for advanced retinoblastoma with concern of tumor invasion into the optic nerve, choroid, or orbit and no hope for salvage of useful vision in the affected eye. Those eyes with secondary glaucoma, pars plana seeding, or anterior chamber invasion are also generally best managed with enucleation.

In the past, most children with unilateral retinoblastoma were managed with enucleation. Those patients with bilateral retinoblastoma usually had the most advanced eye treated with enucleation and the less advanced eye treated with external beam radiotherapy. [200] This management philosophy has been gradually modified with the advent of newer, more conservative but effective methods.[201,202] and [203] There has been a substantial decrease in the frequency of enucleation over recent decades.[204] In a review of 324 consecutive cases of retinoblastoma managed on the Oncology Service at Wills Eye Hospital from 1974 to 1988, Shields and coworkers[204] found that unilateral retinoblastoma was managed with enucleation in 96% of cases from 1974 to 1978, in 86% of cases from 1979 to 1983, and in 75% of cases from 1984 to 1988. A similar decreasing trend was found with bilateral retinoblastoma. [204] The frequency of enucleation is even lower today.

Enucleation involves the gentle removal of the intact eye without seeding the malignancy into the orbit. Special care must be taken to perform all steps in a controlled fashion to avoid globe perforation or compression. Shields and Shields [200] and Shields and colleagues[205,206] have described the surgical technique for enucleation of an eye with retinoblastoma. Because the underlying sclera is thin at the site of muscle insertions, the rectus muscles are handled delicately when the hook is placed flat along the sclera. At the time of optic nerve cutting, scleral or muscle insertion traction sutures are avoided to prevent inadvertent globe perforation. Mild traction with a hemostat on the medial rectus muscle stump is used to lift the globe cautiously to avoid inadvertent lamellar rip of the sclera and cornea, which could threaten the integrity of the eye. Optic nerve snares or clamps should be avoided because they induce more vigorous trauma to the eye and can produce crush artifact in the optic nerve. This artifact can cause difficulty for the pathologist assessing the possibility of retinoblastoma invasion of the optic nerve. The use of minimally curved enucleation scissors is preferred to achieve a long optic nerve section ( Fig. 28-11).

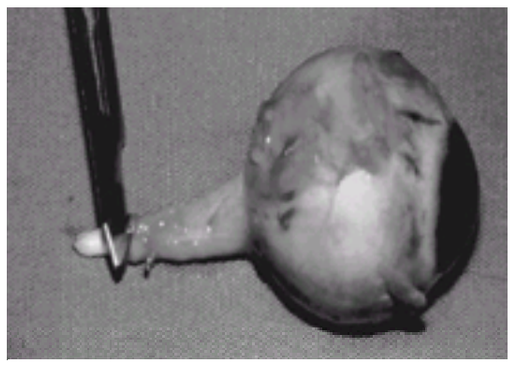

**FIGURE 28-11.** Enucleation and fresh tissue harvesting. A long section of optic nerve is obtained with the globe at enucleation of an eye with retinoblastoma. The posterior aspect of the optic nerve is cut and submitted to pathology separately for analysis of optic nerve invasion.

Historically, an orbital implant was not usually placed after enucleation for retinoblastoma because of potential interference with palpation of the socket and clinical detection of orbital tumor recurrence. More recently, with improved knowledge of the behavior of retinoblastoma and the risks of local orbital recurrence, there is less hesitation for placing an orbital implant. Available orbital imaging modalities, including CT and MRI, allow detailed orbital analysis despite the presence of an implant. The orbital implant provides a more natural cosmetic appearance of the patient's artificial eye, minimizes sinking of the prosthesis, and enables motility of the prosthesis. Orbital implants made of polymethylmethacrylate sphere, coralline hydroxyapatite, bovine hydroxyapatite, or polyethylene are commonly used. [205,207] A tissue wrap is usually provided to these implants so that the four rectus muscles can be anatomically reattached to the implant and provide implant motility with little

resistance in the orbit. Available tissue wraps are many and include povidone iodine-treated human sclera, irradiated human sclera, bovine pericardium, fascia lata, and polyglactin 910 (Vicryl) mesh, among others. The motility implant, when properly placed surgically, has been shown to be well tolerated by children and adults.[205,206,208]

### External Beam Radiotherapy

Retinoblastoma is generally a radiosensitive tumor. External beam radiotherapy is a method of delivering whole-eye irradiation to treat advanced retinoblastoma, particularly when there is diffuse vitreous seeding. The whole-eye and lens-sparing techniques used currently have been shown to improve the eye preservation rate as compared to reported older techniques. The rate of ocular salvage depends on the Reese-Ellsworth stage of the disease at the time of treatment as well as on the availability of focal therapy for limited recurrences. [209,210] Recurrence of retinoblastoma after external beam radiotherapy continues to be a problem and can develop within the first 1 to 4 years after treatment.[211] Tumor recurrence in other studies has also been found to be related to the stage of the disease and to the largest tumor size at the time of treatment.[211,212,213] and [214] Prophylactic radiotherapy to a normal contralateral eye is almost never indicated today. [215]

Little has been written on the visual outcome after external beam radiotherapy for retinoblastoma. Radiation damage to the retina, optic nerve, and lens can be challenging to manage.[216] Patients with macular retinoblastoma have visual outcomes that are dependent on the size of the tumor and the degree of involvement of the fovea.[217] Superimposed amblyopia can pose a problem, and patching therapy should be used if hope for vision remains.

External beam radiotherapy may induce a second cancer in the field of irradiation. The 30-year cumulative incidence for second cancers in bilateral retinoblastoma has been reported to be 35% for patients who received radiation therapy, compared to 6% for those who did not receive radiation. [218] Overall, the cumulative probability of death from second primary neoplasms was reported at 26% at 40 years after bilateral retinoblastoma diagnosis. External beam radiotherapy has been reported to further increase the risk of mortality from second neoplasms. [42] Abramson and Frank[219] found that external beam radiotherapy increased the incidence of second cancers in the field of radiation but did not stimulate second cancers outside the field of irradiation. In their series, patients younger than 12 months were more likely to develop second malignancies after external beam radiotherapy than patients older than 12 months. [219]

### Plaque Radiotherapy

Plaque radiotherapy is a form of brachytherapy in which a radioactive implant is placed on the sclera over the base of a retinoblastoma with the intent of irradiating the tumor transsclerally. The use of plaque radiotherapy is limited to tumors less than 16 mm in base and 8 mm in thickness. Effective treatment requires an average of 2 to 4 days of treatment time to deliver the total dose of 4,000 cGy to the apex of the tumor. Plaque radiotherapy can be used as either a primary treatment or a secondary treatment (Fig. 28-12).[220,221,222] and [223] In 70% of cases, plaque radiotherapy is used as a secondary treatment to salvage a globe after failure of prior treatment, usually failed external beam radiotherapy or chemotherapy. [200,201,202,203,204,205,206,207,208,209,210,211,212,213,214,215,216,217,218,219,220,221,222] and [223] In one series, solitary plaque radiotherapy was used in 91 cases of recurrent or residual retinoblastoma in which the only other option was enucleation. [223] Tumor control and globe salvage were achieved in nearly 90% of these eyes.[223]

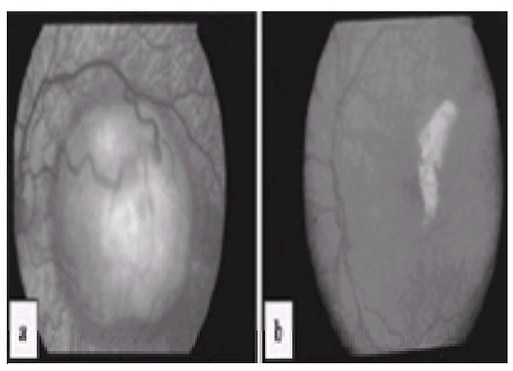

**FIGURE 28-12.** Plaque radiotherapy. **A:** Macular retinoblastoma before plaque radiotherapy. **B:** Regressed retinoblastoma after plaque radiotherapy.

Overall there is nearly a 90% tumor control rate with one application of plaque radiotherapy. [224] Carefully selected retinoblastomas, even juxtapapillary and macular tumors, can be successfully treated with plaque radiotherapy. The visual outcome for the patient varies with tumor size and location as well as associated radiation toxicity, which can include retinopathy or papillopathy. Positive visual outcomes have been reported in 62% of patients; the measured vision was 20/20 to 20/30 in more than one-half of the cases.[220] Radiation retinopathy and papillopathy become clinically manifest at approximately 18 months after irradiation, and these complications are more prominent in children who have been exposed to systemic chemotherapy. In an effort to avoid these problems with chemotherapy-treated patients, the tumor apex dose has been decreased to 3,500 cGy, and radiation plaque therapy is delayed for at least 1 month after the child has discontinued chemotherapy. Innovations with custom design of plaques, especially those for small tumor recurrences, have also assisted in avoiding radiation retinopathy. Because of the use of focal, shielded radiation fields, plaque radiotherapy has not yet been found to be associated with induction of second cancers.

### Laser Photocoagulation

Laser photocoagulation can be used to treat small posterior retinoblastomas using argon laser, diode laser, or xenon arc photocoagulation. Because the tumor size is important to the successful use of this treatment, tumors 4.5 mm or less in base and 2.5 mm or less in thickness with no evidence of vitreous seeds are usually selected.[225,226] The treatment is directed to delimit the tumor and specifically coagulate all blood supply to the tumor. Two or three sessions at 1-month intervals are usually adequate to control most tumors. Use of the indirect ophthalmoscope laser photocoagulation system has greatly improved the facility of laser delivery. [227] With laser treatment of properly selected cases of retinoblastoma, a 70% tumor control rate can be achieved. Recurrences are often treated with plaque radiotherapy. Complications of treatment include transient serous retinal detachment, visually significant retinal vascular occlusion, retinal traction, retinal hole, and preretinal fibrosis.

### Cryotherapy

Cryotherapy is useful for managing equatorial and peripheral small retinoblastomas and is most successful if limited to tumors measuring 3.5 mm or less in diameter and 2.0 mm or less in thickness.[228] Tumor destruction is usually achieved with one or two sessions of triple freeze-thaw cryotherapy at 1-month intervals. Cryotherapy will usually fail if there are overlying vitreous seeds. In these failed cases, plaque radiotherapy is usually used. Complications of cryotherapy include transient serous retinal detachment, retinal tear, localized preretinal fibrosis, and rhegmatogenous retinal detachment. [229]

### Thermotherapy and Chemothermotherapy

Thermotherapy uses ultrasound, microwaves, or infrared radiation to deliver heat to the eye. The heat can be delivered to the whole eye with an attempt to spare the anterior segment,[230] or the heat can be focused on one portion of the eye. The goal is to achieve a temperature of 42 $^{o}$ to 60$^{o}$C, a temperature that is below the coagulative threshold and therefore spares the retinal vessels of photocoagulation. The combination of heat and chemotherapy is termed *chemothermotherapy*, and the combination of heat and radiation is termed *thermoradiotherapy*. Heat has been found to have a synergistic effect with both chemotherapy and radiation therapy for the treatment of systemic and ocular cancers.[231,232]

The selection of the modality of thermotherapy or chemothermotherapy depends on many factors, including tumor size, location, laterality, status of the opposite eye, presence of subretinal fluid and seeds, presence of vitreous seeds, and prior or ongoing chemoreduction. Thermotherapy alone can often be used to effectively treat small retinoblastomas, outside the retinal vascular arcades, measuring 3 mm or less in size without vitreous or subretinal seeds. Thermotherapy alone without chemotherapy may be appropriate. The addition of other factors, such as larger tumors or seeds, often necessitates chemotherapy combined with thermotherapy for

best tumor control.

When using thermotherapy alone, the goal is to heat the tumor to 45 $^{o}$ to 60$^{o}$C, which would leave a gray-white scar at the site. In general, small tumors require approximately 300 MW power for 10 minutes or less, repeated for three times at 1-month intervals ( Fig. 28-13). Tractional and vasoocclusive complications can occur within the retina due to the prolonged heating. When employing chemothermotherapy, the goal is to heat the tumor to 42 $^{o}$ to 45$^{o}$C for 5 to 20 minutes depending on the tumor size and location. Tumors up to 15 mm in base can be adequately treated with chemothermotherapy, especially if the patient is receiving three-agent chemoreduction. The result from chemothermotherapy is a light gray scar with less risk for tractional and retinal vascular problems than is found with thermotherapy alone.[231]

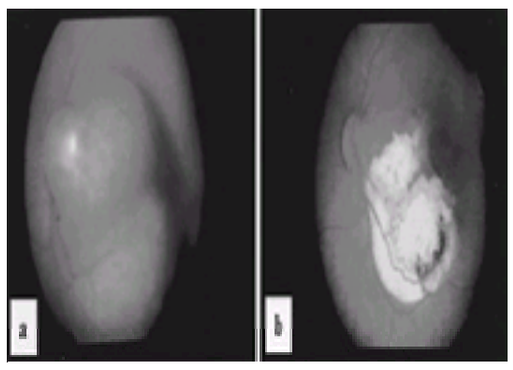

**FIGURE 28-13.** Chemoreduction and chemothermotherapy. **A:** Large macular retinoblastoma overhanging the optic disk. **B:** After chemoreduction and chemothermotherapy, the tumor has regressed to a calcified regressed scar.

There are several different chemothermotherapy protocols, each of which varies in chemotherapeutic agents and methods of delivery. Kaneko and coworkers [276] reported preliminary results using systemic and superselective ophthalmic artery injection of chemotherapy combined with thermotherapy. Murphree and Munier [233] have used a specific protocol of intravenous carboplatin tightly coupled with thermotherapy. Shields and associates [231,234,235 and 236] coupled thermotherapy within 4 hours of a chemoreduction regimen, thereby achieving the benefit of chemotherapy for both tumor reduction and consolidation. This method was more practical for those children with large or multiple tumors simultaneously on a chemoreduction protocol ( Fig. 28-13). If a child is receiving chemoreduction, thermotherapy for tumor consolidation is generally initiated at cycle two or three of the chemoreduction protocol. Thermotherapy is repeated as necessary at each of the remaining chemoreduction cycles until six cycles are completed. Using this method for 188 retinoblastomas, complete tumor control in 86% of the tumors has been achieved. [231] Success of this combined therapy is dependent on careful identification of suitable tumors. Smaller tumors without subretinal fluid or tumor seeds show the best response. In a recent study on the use of thermotherapy and chemothermotherapy for retinoblastoma, tumors less than 3 mm in base responded best with complete control and few complications.[231] Tumors greater than 6 mm in base are at increased risk for recurrence of the main tumor or associated seeds and often require plaque radiotherapy.

The main complication of thermotherapy is focal iris atrophy related to heat effects on the pigmented iris tissue. [231] In some instances, the lens develops a focal paraxial opacity. Chemothermotherapy is especially suited for small tumors adjacent to the fovea and optic nerve in which radiation or laser photocoagulation would possibly induce more profound visual loss. This treatment modality is a time-consuming, tedious process that requires careful observations, recordings, judgments, and treatment adjustments in response to subtle tumor changes.

### Intravenous Chemoreduction for Intraocular Retinoblastoma

Until recently, chemotherapy played only a minor role in the treatment of retinoblastoma. Chemotherapy was only used for patients in whom the disease had spread into the choroid, optic nerve or orbit, or to distant extraocular sites. In the past few years, considerable experience has been gained in the use of chemotherapy for patients with intraocular retinoblastoma involving only the retina. The main objective of the ongoing clinical trials using chemotherapy in localized intraocular retinoblastoma has been to reduce the size of the tumors to an extent that would allow a variety of local surgical modalities such as laser photocoagulation, cryotherapy, or thermotherapy to control the residual disease. Successful management using chemotherapy in combination with local surgical methods can eliminate the use of external beam radiotherapy and, therefore, reduce significantly the risk of development of secondary malignancies and abnormalities of growth of orbital and facial bones associated with radiotherapy.[219,237,238 and 239]

The indications for chemoreduction in intraocular retinoblastoma are not clearly established. The goal of preserving vision in at least one eye while curing children with retinoblastoma makes patients with bilateral retinoblastoma the initial focal point for this form of therapy. A pilot study involving 40 eyes in 31 patients with bilateral disease who were treated with vincristine, teniposide, and carboplatin combined with cyclosporine resulted in a relapse-free rate of 89% in patients who were not previously treated.[240] Relapse in this study was defined as tumor progression requiring either radiotherapy or enucleation. The median follow-up at the time of this report was 2.7 years. In another study of 20 patients who had 54 tumors in 31 eyes, a 2-month chemoreduction program with vincristine, carboplatin, and etoposide was followed by local treatment methods.[241] Enucleation was avoided in all and external beam radiation therapy was necessary in only nine eyes because of diffuse vitreous seeds. Before therapy, the mean tumor base was 12 mm and the thickness 7 mm. Vitreous seeds were present in 14 eyes. After the 2-month chemotherapy regimen, there was no evidence of residual viable tumor in 25 of 54 tumors. There was a mean decrease of 35% in tumor base and nearly 50% decrease in tumor thickness, with resolution of subretinal fluid in 76% of cases. In the 14 eyes that had vitreal seeds, however, only 5 showed 90% to 100% calcification, indicating that different therapeutic modalities must be developed for the treatment of vitreous seeds.

### Chemotherapy for Possible Metastatic Disease

The treatment strategy for patients with intraocular retinoblastoma and extraretinal spread has not been conclusively studied. This includes patients with pathologic evidence of disease with extensive choroidal involvement, evidence of disease extending to the sclera, or disease extending beyond the lamina cribrosa but not yet involving the cut end of the optic nerve. There are conflicting reports about the significance of choroidal invasion. Kopelman and colleagues [242] reported that choroidal invasion was not significantly associated with a fatal outcome. Messmer and colleagues [243] reported that choroidal invasion was a low-risk factor and was not clinically significant when it was the only risk factor. The clinical significance increased considerably when choroidal invasion occurred in combination with other risk factors, such as postlaminar optic nerve involvement, involvement of the optic nerve to the transection line, and late enucleation. Shields and coworkers [244] reported that patients with choroidal invasion with any optic nerve invasion were at high risk for the development of metastases. However, in those patients with choroidal invasion alone the risk for metastases was insignificant. In this study choroidal invasion was not classified as to extent of invasion, although others have reported that the degree of choroidal invasion assessed subjectively was an accurate predictor of survival. [245] In the above studies, some of the patients with choroid invasion with or without optic nerve invasion received radiotherapy, chemotherapy, or both, further clouding the interpretation of true metastatic potential of these risk factors. If significant choroidal invasion is present in the absence of optic nerve invasion, prophylactic adjuvant therapy may be considered. When extensive choroidal invasion is present in combination with optic nerve invasion beyond the lamina cribrosa, prophylactic adjuvant therapy is indicated.

Various chemotherapy regimens have been used for significant deep choroidal, optic nerve, ciliary body, or iris involvement. The combination of cyclophosphamide and doxorubicin has been used.[246] Carboplatin, vincristine, and etoposide for 6 to 18 months has also been recommended. [247,248] Recent retrospective studies suggest that prophylactic chemotherapy in patients with retinoblastoma with some of the above high-risk features can minimize the risk of metastases. [249] There are no randomized studies to recommend a particular regimen, just as there are no randomized studies to identify specific criteria in patients with intraocular retinoblastoma with extraretinal extension who would benefit from chemotherapy.

### Combined Radiotherapy and Chemotherapy

In three separate studies, patients presenting with Reese-Ellsworth eye group V retinoblastoma treated with radiotherapy alone have had 10%, 29%, and 66% of their eyes salvaged.[209,214,250] In patients with bilateral retinoblastoma Reese-Ellsworth eye group V, Kingston and colleagues [251] have shown that two cycles of

chemotherapy in addition to external beam radiation therapy can preserve 70% of the eyes treated (14 of 20 eyes) with a median follow-up of 60 months. The study reporting a 66% salvage rate with radiotherapy alone and the study using chemotherapy in addition to radiotherapy were performed at the same institution; however, the patients in the latter study were reportedly more severely affected. Taken together these data suggest that, for patients with retinoblastoma group V disease, the combination of chemotherapy with external beam radiotherapy may result in a salvage rate superior to that with radiotherapy alone. In a recent study with a median follow-up of 13 months, chemotherapy alone consisting of six cycles of carboplatin, vincristine, and etoposide resulted in the salvage of only 50% of the eyes with groups IV and V disease without requiring external beam radiotherapy or enucleation. [248] In the same study, chemotherapy eliminated the need for external beam radiotherapy or enucleation in all 39 eyes with groups I, II, and III disease ( Table 28-2).

| Reese-Ellsworth Group | EBRT alone (%) Ellsworth[204] (1963–1972) | EBRT + salvage treatment (%) Hungerford[209] (1970–1985) | Chemoreduction[a] + adjuvant treatment[b] (%) Shields[203] (1994–1998) |
|---|---|---|---|
| I | 91 | 100 | 100 |
| II | 83 | 64 | 100 |
| III | 82 | 82 | 100 |
| IV | 62 | 43 | 100 |
| V | 29 | 66 | 78 |

Chemoreduction regimen (6 cycles) for intraocular retinoblastoma

| Day | Vincristine[c] | Etoposide[d] | Carboplatin[e] |
|---|---|---|---|
| 3 | + | + | + |
| 1 | — | + | — |

EBRT, external beam radiotherapy.
[a]Using vincristine, etoposide, and carboplatin.
[b]Laser photocoagulation, cryotherapy, thermotherapy, chemothermotherapy, plaque radiotherapy, EBRT.
[c]Vincristine, 1.5 mg/m² (0.05 mg/kg for children aged ≤36 mo and maximum dose ≤2 mg).
[d]Etoposide, 150 mg/m² (5 mg/kg for children aged ≤36 mo).
[e]Carboplatin, 560 mg/m² (18.6 mg/kg for children aged ≤36 mo).

**TABLE 28-2. PERCENTAGE OF GLOBES SALVAGED USING EXTERNAL BEAM RADIOTHERAPY ALONE, EXTERNAL BEAM RADIOTHERAPY AND SALVAGE TREATMENT, AND CHEMOREDUCTION AND FOCAL ADJUVANT TREATMENT**

From the above data it is clear that chemoreduction is an effective initial measure for selected children with intraocular retinoblastoma. Retinal tumor and seed recurrence remain a worrisome problem with chemoreduction, however.[203] Seeds in the vitreous or subretinal space can recur in approximately 30% of eyes and enlarge to a visual and life threatening state. Chemotherapy regimens used have included carboplatin, etoposide, and vincristine. Cyclosporine has been used with the above regimen in an attempt to improve results by reversing multidrug resistance. [252,253] Use of chemotherapy is not without concern, especially in patients with bilateral retinoblastoma who have a higher incidence of second malignancies. The use of etoposide, an epipodophyllotoxin, has been associated with second malignancies in patients with leukemia and non-Hodgkin's lymphoma. However, the schedule and the cumulative dose of etoposide used in most of the treatment regimens for retinoblastoma are different from those implicated in the incidence of second malignancies. [254] Other side effects of concern are transient bone marrow suppression with a risk for infection.

### Treatment of Systemic Retinoblastoma

Treatment of extraocular retinoblastoma requires a combined therapeutic approach using both chemotherapy and radiotherapy. Scleral involvement, orbital or bony involvement, involvement beyond the cut end of the optic nerve, metastatic disease involving brain or other sites, and trilateral retinoblastoma all require such an aggressive combined therapeutic approach. Central nervous system involvement generally carries a poor prognosis.

Many different agents have been used to treat systemic retinoblastoma. One regimen used is the three-drug regimen including vincristine, carboplatin, and etoposide, similar to the chemoreduction regimen mentioned above but with a much longer course of 6 to 18 months, depending on the clinical response. [203,241] Others have found favorable results with similar chemotherapy regimens for extraocular retinoblastoma. [255,256,257,258] and [259] White[260] has reported on chemotherapy for retinoblastoma and recently advocated cyclophosphamide, etoposide, and vincristine as well as the support of peripheral stem cell rescue in multiple sequential courses for metastatic retinoblastoma.

### Orbital Exenteration

Orbital exenteration is rarely used for retinoblastoma management in the United States, as most retinoblastoma patients present with no evidence of extraocular invasion.[261] Exenteration is most often used for orbital recurrence after the child has received a maximum acceptable dose of irradiation and chemotherapy. In other countries, patients may present with more advanced retinoblastoma, including orbital involvement. For these patients, exenteration, chemotherapy, and external beam radiotherapy are crucial for survival. Use of more advanced exenteration techniques, such as the eyelid-sparing exenteration, allows for rapid healing of the wound. [262]

### Trilateral Retinoblastoma

*Trilateral retinoblastoma* is a term to describe the association of bilateral retinoblastoma and neuroblastic tumor in the pineal gland or other midline structures. A report from de Potter and coworkers[263] revealed that this tumor occurs in children aged 4 years of age or younger. MRI or CT is essential to the diagnosis. The disease is highly fatal despite aggressive treatment with chemotherapy, radiation therapy, and gamma knife therapy. Longer survival has been correlated with earlier tumor diagnosis in asymptomatic patients. Trilateral retinoblastoma is a major cause of mortality in children within the first 5 years after diagnosis of bilateral retinoblastoma.[264] No case of pinealoblastoma was observed in 147 children treated with initial chemoreduction who were followed for 1 to 4 years. Although follow-up is limited, it is tempting to speculate that chemoreduction may reduce the risk for development of pinealoblastoma. [265]

## HISTOPATHOLOGIC CHANGES ASSOCIATED WITH TREATMENT

Enucleation of eyes from patients with retinoblastoma treated with different modalities that either do not eradicate the tumor or result in major treatment complications allows the study of effects of therapy on the tumor and the ocular structures. Clinically, three types of regression patterns have been described in tumors that have undergone treatment: type 1 (cottage cheese), type 2 (fish flesh) or type 3 (combined).

In one study, five patients with sporadic bilateral retinoblastoma underwent planned enucleation of their functionally blind eye after two, three (in two patients), four, and six courses of primary chemotherapy with carboplatin, etoposide, cyclophosphamide, and vincristine. The eyes were examined histopathologically, using light microscopy and immunohistochemical analysis with proliferation markers. One patient had a type 1 (cottage cheese) regression and four patients had either a type 2 (fish flesh) or a type 3 (combined) regression pattern. Histopathologic examination revealed complete tumor necrosis with calcification ( Fig. 28-14) in one patient with type 1 regression after three courses of chemotherapy and in one patient with type 3 regression after four courses of chemotherapy. The remaining three patients with type 2 or type 3 regression had histological evidence of actively proliferating tumor cells after two, three, and six courses of chemotherapy. This report confirms histopathologically the clinically described efficacy of primary chemotherapy in the treatment of retinoblastoma. The necessity of careful observation and the use of ancillary treatment whenever there is not complete tumor regression (type 2 and 3 regression patterns) is, however, underscored. [266] In another study, photoreceptor differentiation was observed in 17 of 42 enucleated eyes containing viable tumor following radiotherapy. Complications of external beam radiation include massive necrosis of the retina with associated hemorrhage (Fig. 28-14). Patients with retinoblastoma that have undergone chemotherapy and radiotherapy sometimes are left with tumors with large areas of fossilized cells and calcification with areas of photoreceptor differentiation similar to retinocytoma. Whether these cases represent chemotherapy or radiotherapy resistance of a focus of retinocytoma or differentiation induced by treatment has not been elucidated. [267]

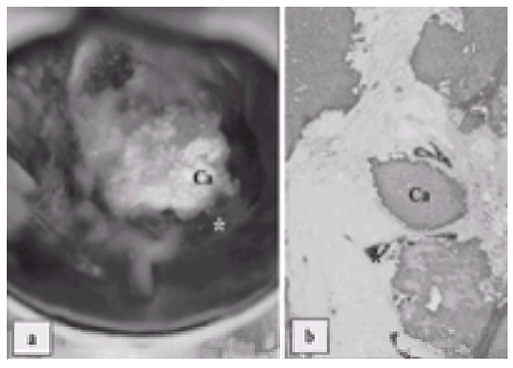

**FIGURE 28-14. A:** Gross photograph of an autopsy eye in a patient with regressed retinoblastoma for more than 6 years after chemotherapy and radiotherapy. The patient died of complications of chemotherapy (cardiomyopathy). Notice the cottage cheese appearance (type 1 regression pattern) of the calcified (Ca) tumor associated with proliferation of retinal pigment epithelium ( *asterisk*). **B:** Microscopic photograph of the same eye as **(A)** showing the calcified tumor (Ca), proliferation of retinal pigment epithelium (*asterisk*), and admixed glial tissue. (Hematoxylin and eosin; original magnification 20×.)

## FUTURE DIRECTIONS

### Subconjunctival Chemoreduction for Intraocular Retinoblastoma

To avoid the toxicity of systemic administration of chemotherapy, there is increasing interest in local delivery of these drugs to achieve chemoreduction for intraocular retinoblastoma. Studies in animal models show that carboplatin penetrates the sclera into the vitreous cavity, allowing for effective dosages within the eye with minimal toxicity.[268,269 and 270] Local subconjunctival injection of carboplatin under protocol in humans as both a secondary and a primary treatment have been used [271] (C.L. Shields and J.A. Shields, *unpublished data*). Within 3 to 4 weeks, tumor regression is usually noted, but the response may not be long term. Further investigations are necessary to determine the efficacy of local application of chemotherapy.

### Chemotherapy to Prevent and Treat Metastases

The development of innovative modalities in the treatment of retinoblastoma has been hampered by the lack of suitable animal models of this uniquely human disease. Recently murine models of retinoblastoma using human xenografts in the vitreal space that mimic both metastatic and nonmetastatic disease [272] as well as transgenic mouse models of retinoblastoma[273] have been developed. These newer animal models will aid in the testing of new therapeutic approaches to retinoblastoma.

### Gene Therapy

An alternative approach to local chemoreduction without the side effects of systemic chemotherapy is gene therapy. One form of gene therapy, *suicide gene therapy*, uses the delivery of the herpes simplex thymidine kinase gene by a replication-defective adenoviral vector injected directly into the tumor. Ganciclovir, a nucleoside analog, is then administered intravenously to the patients. The expressed thymidine kinase phosphorylates the ganciclovir within the tumor cells. The resulting nucleotide analog is a potent inhibitor of DNA synthesis and causes the death of the dividing tumor cells. Nondividing cells are unaffected. The safety of this form of suicide gene therapy has been demonstrated in patients with brain tumors.[274] Effective reduction of retinoblastoma tumors in a mouse model of the disease has also recently been demonstrated.[275] A phase I clinical trial has been approved and is currently testing the safety of this approach in children with retinoblastoma.

### International Cooperative Studies

Because retinoblastoma is a very rare disease, clinical trials addressing basic diagnostic and therapeutic questions are very difficult to perform. Recently, the American College of Surgeons Oncology Group and the Children's Oncology Goup have formed Retinoblastoma Study Groups to address therapeutic questions concerning the treatment of retinoblastoma.

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# 29

# TUMORS OF THE LIVER

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MILTON J. FINEGOLD



## EPIDEMIOLOGY

Of all liver masses in children, approximately two-thirds are malignant. Malignant liver tumors account for approximately 1.1% of all childhood tumors according to the Surveillance, Epidemiology, and End Results (SEER) program cancer registries, with an annual incidence rate of 1.5 cases per million children younger than 15 years.[1] From these data, it has been calculated that approximately 100 to 150 new cases of liver cancer in children develop in the United States each year. Of the malignant liver tumors of childhood, hepatoblastoma accounts for approximately two-thirds. A listing of the various types of hepatic tumors, both benign and malignant, along with the typical ages of presentation is shown in Table 29-1. Eleven separate series totaling 1,256 primary benign and malignant liver tumors in children were reviewed in Weinberg and Finegold,[2] and of these, 43% were hepatoblastoma, 23% hepatocellular carcinoma (HCC), 13% benign vascular tumors, 6% mesenchymal hamartomas, 6% sarcomas, 2% adenomas, 2% focal nodular hyperplasia, and 5% other tumors. Although the salient features of the less common forms of liver cancer in children are discussed briefly, this chapter focuses primarily on hepatoblastoma and HCC, which together account for the majority of malignant liver tumors in children.

| Age | Benign | Malignant |
|---|---|---|
| Infancy (0–1 yr) | Hemangioendothelioma<br>Mesenchymal hamartoma<br>Teratoma | Hepatoblastoma, especially small cell undifferentiated<br>Rhabdoid tumor<br>Yolk sac tumor<br>Langerhans' cell histiocytosis<br>Megakaryoblastic leukemia<br>Disseminated neuroblastoma |
| Early childhood (1–3 yr) | Hemangioendothelioma<br>Mesenchymal hamartoma<br>Inflammatory myofibroblastic tumor | Hepatoblastoma<br>Rhabdomyosarcoma |
| Later childhood (3–10 yr) | Angiomyolipoma | HCC<br>Embryonal sarcoma (undifferentiated)<br>Angiosarcoma |
| Adolescence (10–15 yr) | Adenoma<br>Biliary cystadenoma | Fibrolamellar HCC<br>Hodgkin's disease/lymphoma<br>Leiomyosarcoma |

HCC, hepatocellular carcinoma.

**TABLE 29-1. NEOPLASIA OF THE LIVER IN CHILDREN ACCORDING TO USUAL AGE OF PRESENTATION**

Most cases of hepatoblastoma occur in infants or very young children. The observed incidence rate of malignant liver tumors in infancy is 11.2 per million and decreases throughout childhood.[1] In a recent Pediatric Oncology Group (POG) series of 106 hepatoblastomas accrued on biologic studies, the mean age of diagnosis was 19 months and the median age was 16 months, consistent with that of previous reports.[3,4] Only 5% of cases occurred in children older than 4 years of age. Hepatoblastoma has been reported in adults, although this is rare. [5,6] Hepatoblastoma is more common in males with a reported male to female ratio of 1.4:1.0 to 2.0:1.0.[2,3 and 4]

HCC occurs primarily after age 10 years and is the most common hepatic malignancy of adolescence. The clearest pathogenetic factor is hepatitis B virus. When hepatitis B infection is acquired after the prenatal period, it may take 20 years to demonstrate its carcinogenic effect; however, perinatally acquired hepatitis B virus has been associated with hepatocarcinoma in very young. [7] Similar to hepatoblastoma, HCC demonstrates a male predominance, except for the fibrolamellar variant, which is seen in equal frequency in males and females.[8,9 and 10] Occasionally, malignant tumors are seen that have features of both hepatoblastoma and HCC ( Fig. 29-1). There are several suggestions that the incidence of liver tumors in children is increasing in the United States; this is discussed in more detail below.

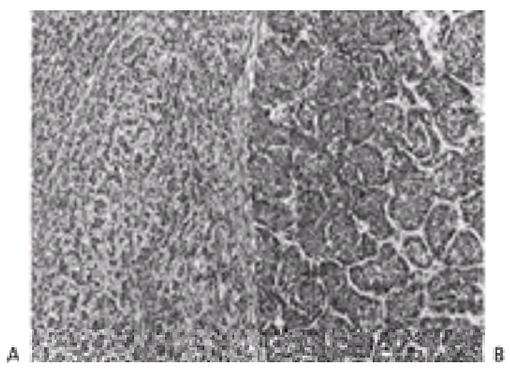


**FIGURE 29-1.** Composite tumor from a 15-year-old with perinatally acquired hepatitis B infection. Well-differentiated fetal hepatoblastoma is present in **(A)** and a macrotrabecular hepatocarcinoma in **(B)**. Hepatitis B core antigen was abundant in the hepatoblastoma and in the nontumoral hepatocytes affected by chronic hepatitis. Only the carcinoma was positive for a-fetoprotein.

In the Far Eastern regions of the world, the incidence of liver cancer has historically been higher than that in the United States. The International Agency for Research on Cancer reported that during the 1970s the incidence of liver cancer in Hong Kong, Shanghai, Taiwan, and Fiji was 4.0 per million children younger than 15 years; this was tenfold higher than in western countries, primarily due to the high carrier rate for hepatitis B in these countries. [11] In Taiwan in 1988, 80% of primary liver tumors in children were reported to be HCC.[12]

During the past two decades, data from several sources have suggested an increase in the number of cases of hepatoblastoma. The SEER data reveal an average 5% annual percent increase in the incidence of hepatoblastoma from 1972 to 1992. [13] In the period from 1979 to 1981, liver cancers represented 2% of all cancers in

infants younger than 1 year, whereas a decade later, liver cancers represented 4% of all cancers in infants. [14] The Manchester, England, Tumor Registry observed an increased incidence of hepatoblastoma from 0.4 to 1.0 per million.[11] The SEER data have also revealed a significant increase in the incidence of HCC in the overall population, including both children and adults, from the period 1991 to 1995 compared to 1976 to 1990. [15] This is in contrast to a marked decrease in the incidence due to the introduction of universal hepatitis B vaccination in Taiwan, in which hepatitis B historically has played a major role in the development of HCC. [16]

Mounting evidence suggests an association of hepatoblastoma with prematurity, which may account for part of the observed increase in hepatoblastoma overall as survival rates have increased among premature infants. Ikeda and colleagues [17,18] observed that in Japan, hepatoblastomas account for 58% of all malignancies occurring in surviving premature infants weighing less than 1,000 g at birth. Further analysis of the Japanese Children's Cancer Registry data revealed that 15 of 303 (5%) hepatoblastomas between 1985 and 1995 occurred in postpremature infants weighing less than 1,500 g. The relative risk for hepatoblastoma increased inversely with birth weight. The relative risk of infants weighing less than 1,000 g at birth was 15.64 compared to 2.53 for infants weighing 1,000 to 1,499 g and 1.21 for infants weighing 2,000 to 2,499 g.[19] These data have implications for the need to determine the specific factors related to prematurity which contribute to hepatic tumorigenesis as well as the need for surveillance of the survivors or extreme prematurity.

## ETIOLOGY

The causes of most liver tumors, similar to other types of childhood cancer, are unknown. Hepatoblastoma occurs in association with several well-described cancer genetic syndromes. HCC often occurs after the development of cirrhosis, which may have both genetic and environmental contributions.

A number of associations have been observed of liver tumors in children with genetic syndromes. These are summarized in Table 29-2. In the absence of a recognizable familial cancer syndrome, hepatoblastoma has been observed in siblings. [20,21 and 22]

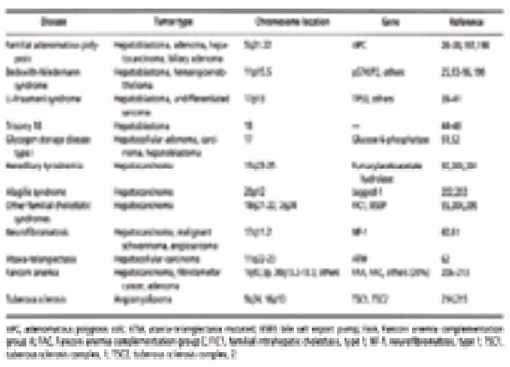

**TABLE 29-2. CONSTITUTIONAL GENETIC SYNDROMES LEADING TO LIVER TUMORS**

Beckwith-Wiedemann syndrome is associated with a risk of embryonal tumors.[23,24] Data recently reported from the National Cancer Institute's Beckwith-Wiedemann support group indicate a relative risk of hepatoblastoma as 2,280, higher than that for other embryonal tumors, including Wilms' tumor. [25] The recognition of physical stigmata of Beckwith-Wiedemann syndrome in an infant should prompt surveillance efforts for detection for embryonal tumors by means of serial abdominal sonography and serum a-fetoprotein (AFP) measurements.

The association of hepatoblastoma with familial adenomatous polyposis (FAP) was first reported by Kingston. [26,27] Several additional reports of case studies and small series have further documented the association of hepatoblastoma and adenomatous polyposis. [28,29,30,31,32 and 33] This syndrome is caused by germline mutation of the adenomatous polyposis coli (APC) gene.[34] Giardiello and colleagues [35] have estimated a relative risk of 800 of hepatoblastoma in children in FAP families compared to the general population risk. There are no definitive differences in age range, histologic type, or outcome in hepatoblastomas associated with FAP. FAP has also been implicated in the pathogenesis of some cases of hepatocellular adenoma, HCC, and fibrolamellar carcinoma, and it has been hypothesized that mutation of the APC may confer a general low-level predisposition to tumorigenesis in the liver dependent on other environmental or developmental factors. [36,37 and 38]

Although liver tumors are not among the tumor types frequently associated with Li-Fraumeni syndrome, two Li-Fraumeni kindreds reported from Japanese series having underlying mutations of the *TP53* gene have a family member with hepatoblastoma.[39,40] In addition, Lack and colleagues [41] reported a case of undifferentiated (embryonal) sarcoma of the liver in a Li-Fraumeni syndrome kindred. Mutation of the *TP53* gene is not, however, thought to be a major contributing factor in sporadic hepatoblastoma.[42,43]

Trisomy 18 has been observed in several patients with hepatoblastoma, one of whom developed multiple primary tumors. [44,45,46,47 and 48] Hepatoblastoma has also been reported in Prader-Willi syndrome.[49]

Almost all cases of hepatoblastoma, even those that occur in association with predisposition syndromes, occur in the setting of normal hepatic function and histology. HCC, except for the fibrolamellar variant, occurs in various syndromes that produce cirrhosis or other chronic damage to the liver parenchyma.

Children with hereditary tyrosinemia type 1 (fumarylacetoacetate hydrolase deficiency) have a very high incidence of HCC. [50] Glycogen storage disease type I is associated with the development of adenomas and occasionally HCC.[51] Two adolescents aged 12 and 19 with glycogen storage disease type 1A have also been reported to have developed hepatoblastoma.[52] Hepatic adenomas have been observed in type IV glycogen storage disease, [53] and carcinomas have been reported in type III.[54] Alagille syndrome and other familial cholestatic syndromes are also associated with the development of HCC. [55,56 and 57] Many but not all of the latter are also associated with liver dysfunction and cirrhosis of the liver.

$Alpha_1$-antitrypsin deficiency has been associated with an increased incidence of HCC and cholangiocellular carcinomas, although tumors do not occur until late adulthood.[58,59]

Liver tumors of various types are also reported in association with neurofibromatosis, tuberous sclerosis, and ataxia-telangiectasia. [60,61 and 62] In Fanconi's anemia, prolonged use of anabolic steroids such as oxymetholone methyltestosterone is associated with development of hepatic tumors, both benign and malignant. [63] In some cases, tumor regression has been observed with the withdrawal of steroids. The several cases of hepatic tumors in patients with Fanconi's anemia who are treated with anabolic steroids demonstrate how a genetic defect in DNA repair coupled with an exogenous agent may contribute to the development of neoplasia.

Environmental factors have been implicated in hepatoblastoma. Buckley and colleagues [64] reported an association with certain occupational exposures in fathers of children with hepatoblastoma. These include excess exposures to metals such as in welding and soldering fumes (odds ratio, 8.0), petroleum products, and paints (odds ratio, 3.7). A prenatal exposure to acetaminophen in combination with petroleum products has also been noted in association with hepatoblastoma. [65]

Hepatitis B virus has historically played a major role in the development of HCC worldwide. In areas in which hepatitis B virus is endemic, HCC is a common malignancy, and a high rate of hepatitis B surface antigenemia has been observed. Perinatally acquired hepatitis B has also been demonstrated to have integrated into the genome in tumors from children with no clinical signs of present or past hepatitis B infection. [66] Hepatitis C has been implicated to a lesser extent than hepatitis B in the development of HCC in adults, but not in children.

With the intriguing observation in the past decade of increasing incidence of hepatoblastoma in premature infants, we may speculate about possible environmental factors acting in conjunction with prematurity to stimulate tumor development. Prenatal, neonatal, and perinatal factors may play a role, although it is unclear whether this relates to exposures in the neonatal period or other medical complications of either mother or neonate, or perhaps an altered pathway of normal hepatic development that is disrupted by premature birth. Anecdotal reports of hepatoblastoma with oral contraceptives or other use of hormones as well as the development

of hepatoblastoma in infants with fetal alcohol syndrome argue for events early in organogenesis that may be contributory. [67,68 and 69]

Parenteral nutrition in infancy has been associated with the development of HCC in childhood. [70,71] How much of role parenteral nutrition, which is clearly lifesaving for many premature infants, plays in the observed increase in the subsequent development of liver cancer in premature infants will await additional epidemiologic studies.

HCC has also been reported as a second malignancy in patients with Wilms' tumor treated with hepatic irradiation, presumably due to radiation-induced hepatic changes.[72] HCC has also been seen after treatment for acute lymphoblastic leukemia treated with prolonged use of methotrexate associated with hepatic inflammation and damage.[73,74]

## ACQUIRED GENETIC CHANGES IN TUMORS

Karyotyping of hepatoblastomas has revealed a recurrent pattern of chromosomal abnormalities. The most common karyotypic changes are extra copies of entire chromosomes (trisomies), sometimes in conjunction with other complex structural changes and often in association with double-minute chromosomes.[75,76,77,78 and 79] In a single tumor, several clones may be observed, with differing numbers of trisomies suggesting clonal evolution with the addition of extra chromosomes. Trisomies of chromosomes 2 and 20 have each been reported most commonly, and each of these trisomies has been reported as a sole karyotypic event, suggesting that they may represent an early stage of tumor evolution.[75,76 and 77] Trisomy of chromosome 20 and duplication of the long arm of chromosome 20 have been also observed in rhabdomyosarcoma, suggesting a link between these two embryonal tumors, both of which are associated also with losses at the Beckwith-Wiedemann syndrome locus.[80,81 and 82] Trisomy of chromosome 8 is also common, and other trisomies are seen with lesser frequency. Occasional losses of entire chromosomes are seen and these, too, are not random. Figure 29-2 displays the frequency of both chromosomal gains and losses in a series of 42 hepatoblastomas with abnormal karyotypes. [83] The clinical significance of these trisomies is at present unknown, although one recent study using comparative genomic hybridization has suggested that chromosomal gains at chromosome 8 and 20 may be associated with an adverse prognosis.[84] A unique translocation has been reported in undifferentiated small cell hepatoblastoma,[85] a variant associated with a poor prognosis, although this cytogenetic variant has not been reported in repeated cases. Interestingly, cytogenetically visible chromosomal aberrations of chromosome 5q, the locus for the APC gene, or 11p15, the locus for Beckwith-Wiedemann syndrome, have not been reported in sporadic cases of hepatoblastoma. Likewise, trisomy 18, despite its association with hepatoblastoma when present constitutionally, is not a feature of sporadic tumors.

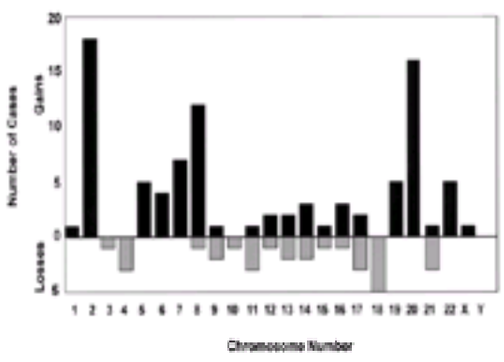


**FIGURE 29-2.** Distribution of numerical abnormalities in a series of 42 hepatoblastoma karyotypes. The most common abnormalities included trisomy of chromosomes 2, 20, and 8. Chromosomal losses occur less commonly. Loss of chromosome 18 has been observed in five cases. (From Schneider N, Tomlinson G. Cytogenetics in a series of hepatoblastoma. In preparation, 2001.)

In 1998 Schneider and colleagues[86] described a translocation, t(1;4)(q12;q34), occurring in four cases of hepatoblastoma, all male and high-stage tumors. Since then others have also reported this translocation. [78,79,87] In all reported cases, this translocation has been associated with other chromosomal changes including trisomies of 2, 8, 20. The breakpoint of this translocation has not yet been characterized. The region involved on chromosome 1 is a region of heterochromatin with few structural genes; the region on chromosome 4 contains multiple genes involved in cellular growth and is also one of several genomic sites at which the hepatitis B virus is known to integrate.[88] The distal region of chromosome 4 is also rearranged in 10% of HCCs. [89,90 and 91]

Several acquired genetic changes are known to occur in hepatoblastoma, which may be shared by other embryonal tumors. [80,81,92] Loss of heterozygosity at chromosome 11p15 is well described as a common feature in embryonal tumors, including Wilms', hepatoblastoma, and rhabdomyosarcoma. [82] It is thought that an important tumor suppressor gene or growth factor gene located at 11p15.5 is a factor in the development of embryonal tumors. It has been generally assumed but not proven that the critical gene lost is the same gene that is responsible for Beckwith-Wiedemann syndrome. Several genes of interest in the study of embryonal tumors and Beckwith-Wiedemann syndrome are located at this chromosomal locus. The p57KIP2 gene at 11p15.5 is a regulator of cellular proliferation and has been shown to be mutated in some families with Beckwith-Wiedemann syndrome.[93,94,95 and 96] Although not mutated in hepatoblastoma, the p57K1P2 gene may be aberrantly expressed in hepatoblastoma.[97] The insulin-like growth factor 2 gene is preferentially expressed from the father in normal tissue. In tumor tissue the parental allele specific expression is variable, however, suggesting that disruption of normal expression pattern may be involved in tumorigenesis. The H19 gene, also at 11p15.5, shows the opposite pattern of expression of parental alleles, although the role of this gene in tumorigenesis is unclear. [98] An intriguing phenomenon observed is that when losses of genetic material at 11p15.5 occur, the losses are always of maternal origin. This suggests that the imprinting of genes, or differential function depending on parental origin, may have a role in the pathogenesis of hepatoblastoma. [99] In hepatoblastomas that do not demonstrate loss of heterozygosity at 11p15.5, the differential expression of genes is lost with subsequent expression from both parental alleles. [100]

Acquired mutations of the APC gene have been reported in a few cases of hepatoblastoma and have not been reported in other embryonal tumors. [101] Several recent studies, however, have focused on abnormalities of b-catenin, whose degradation is regulated by APC. b-Catenin is more commonly altered in hepatoblastoma than is the APC gene itself. A study from Germany has shown that 48% of hepatoblastomas are associated with mutation of the b-catenin gene. [102] The importance of mutation of this gene was confirmed in a second series from Taiwan.[103] Nuclear localization of b-catenin in hepatoblastomas, especially more aggressive tumors, is different from that of normal hepatocytes, which have only plasma membrane localization. [104] These observations suggest that alterations of the wnt signaling pathway play a crucial role in the malignant transformation in immature hepatic cells.

Among the other liver tumors, various cytogenetic changes have been reported. Multiple reports of mesenchymal hamartoma have demonstrated a recurring translocation with a breakpoint at chromosome band 19q13.4. [105,106 and 107] None of the sarcomas observed in association with or following a mesenchymal hamartoma has been shown to have the 19q13.4 breakpoint. A unique karyotype that is near triploid and near hexaploid has been reported in undifferentiated sarcoma, although this has not been observed in additional cases. [108]

A specific *TP53* mutation has been reported in HCC in association with aflatoxin exposure. [109] One study reported a hot-spot mutation of *TP53* in a series of hepatoblastomas.[110] However, other studies of *TP53* in hepatoblastoma have failed to demonstrate that mutations occur frequently. [42,111] In HCC, the presence of a *TP53* mutation or the presence of serum antibody to TP53 is associated with a shorter survival. [112,113]

## PRESENTATION AND INITIAL DIAGNOSTIC ASSESSMENT

Most liver tumors present with an asymptomatic abdominal mass palpated either by a parent or pediatrician. Abdominal pain, weight loss, anorexia, nausea, and vomiting may be present, particularly in advanced disease. Jaundice is rare; however, it is seen in some cases of rhabdomyosarcoma. A presenting feature occasionally seen in males is pseudoprecocious puberty. [4] Infants with hemangioendothelioma may present with signs and symptoms of congestive heart failure.

Presurgical assessment often initially begins with a plain film or ultrasonography, which reveals a right upper quadrant mass. Calcifications on x-ray are seen only in a minority of cases and are nonspecific so that plain films are of very limited value in characterizing hepatic masses. Sonography is useful for the initial detection of

masses, as increased echogenicity is suggestive of malignant disease. The value of sonography is increased when accompanied by Doppler flow studies to assess tumor vascularity. However, sonography is limited in defining tumor margins to definitively establish resectability. To accurately define the extent of disease, computerized tomographic (CT) scanning[114] or enhanced magnetic resonance imaging[115] is needed. Figure 29-3 demonstrates the delineation of a small resectable hepatoblastoma by CT. Magnetic resonance imaging of an unresectable HCC is shown in Figure 29-4.

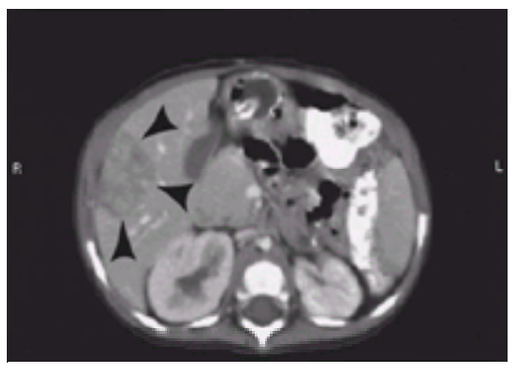

**FIGURE 29-3.** Computed tomography scan of a low-stage hepatoblastoma, demonstrating clear tumor margins (*arrowheads*). This tumor was picked up initially by periodic screening with sonography in a patient with Beckwith-Wiedemann syndrome. (Courtesy of Children's Medical Center, Dallas.)

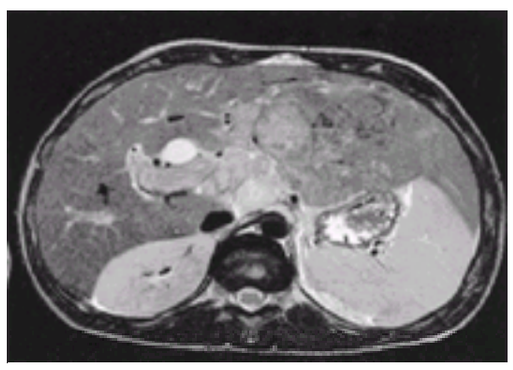

**FIGURE 29-4.** Magnetic resonance imaging study of a hepatocellular carcinoma with extensive involvement of both lobes as well as bulky lymphadenopathy. In this T2-weighted image, tumor involvement of both right and left lobes is visualized as lighter areas compared to normal liver parenchyma. (Courtesy of Dr. Jeanne Dillenbeck, Children's Medical Center, Dallas.)

By far, the vast majority of metastases present at diagnosis occur in the lungs. Therefore, CT imaging of the chest is essential. Bone lesions have been reported but it is unclear whether these represent true metastases or areas of demineralization.[116] Metastasis to the brain has been reported but is extremely rare.[117,118] Bone marrow involvement has only very rarely been observed, and current therapeutic protocols do not call for bone marrow evaluation.

Presurgical laboratory assessment should include a complete blood count. Anemia may be seen and is usual mild and normochromic normocytic. Thrombocytosis is seen in approximately one-fifth of cases[4] and is related to thrombopoietin production by tumor in some, but not all, cases.[119,120 and 121] It has also been suggested that thrombocytosis is mediated by interleukin 6.[122] Liver enzymes and bilirubin are usually normal or only mildly elevated. Human chorionic gonadotropin b is produced by some hepatoblastomas, and substantial elevations result in physical signs of precocious puberty.[123,124] Hypertension secondary to a renin-secreting mixed hepatoblastoma has been reported.[125]

Measurement of AFP is essential and is markedly elevated in more than 90% of hepatoblastomas and in many cases of HCC. The AFP is usually normal, however, in the fibrolamellar variant of HCC as well as in most benign liver tumors. However, we have seen examples of significantly elevated AFP in cases of infantile hemangioendothelioma and mesenchymal hamartoma, which have misled clinicians into treating for hepatoblastoma without confirmatory diagnostic biopsy. AFP is the major protein produced by the fetal liver and is thus produced in high amounts in the newborn. In the normal-term infant the AFP level can be as high as 100,000 ng per mL or greater. The half-life of AFP is 5 to 7 days and levels fall throughout the first several months of life. By age 1 year the AFP level is less than 10 ng per mL.[126] In infants younger than 1 year with hepatoblastoma it may be initially difficult to distinguish the components of elevated AFP from normal liver versus malignant tumor, however, the AFP is a useful tumor marker to both assess response to therapy as well as to monitor for disease recurrence.[127,128] After a complete resection, AFP levels should decline substantially and approach normal ranges within several weeks. Failure to do so may indicate residual disease. It is possible to fractionate the malignant and nonmalignant forms of AFP by immunoelectrophoresis; however, this is not a generally available clinical laboratory procedure.[129,130] Although of potential diagnostic interest, this is not important in the monitoring of AFP levels after the diagnosis in a young infant, because levels should only trend downward if treatment is effective, with a rise in AFP raising concern of recurrent or progressive disease.

As part of the workup of any child with a liver tumor, care should be taken to review neonatalhistory for the presence of features such as high birth weight, hemihypertrophy, macroglossia, umbilical hernia, or ear pits, which would suggest Beckwith-Wiedemann syndrome. In addition, inquiry as to family history of tumors is essential, particularly to ascertain a family history of early-onset colon cancer or colonic polyps or other manifestation of Gardner's syndrome.[131,132] Although recognition of these syndromes would not change the clinical management of the primary tumor, their recognition would have implications for following patients long-term. Patients with Beckwith-Wiedemann syndrome are at potential risk for second embryonal malignancies. Children with hepatoblastoma from polyposis kindreds would be at risk for colon cancer as young adults as well as desmoid tumors and other extracolonic manifestations throughout childhood.

## STAGING

The conventional POG/CCG staging system is shown in Table 29-3.[133,134] As with other solid tumors, *stage I* is defined as a tumor completely resected at diagnosis. *Stage II* refers to resection with microscopic residual disease. *Stage III* indicates gross residual disease, including involvement of local lymph nodes and inability to resect the primary tumor. *Stage IV* tumors are those with distant metastases.

Stage I (favorable histology) tumors are those that are completely resected and have a typical histology of a purely fetal histologic pattern with a low mitotic index (<2 per 10 high-power fields).
Stage I (other histology) tumors are completely resected tumors with a histologic picture other than purely fetal with low mitotic index.
Stage II tumors are grossly resected tumors with evidence of microscopic residual. Such tumors are rare, and patients with this stage have not fared differently from those with stage I tumors in previous protocols. Resected tumors with preoperative (intraoperative) rupture are classified as stage II.
Stage III (unresectable) tumors are those that are considered by the attending surgeon to be not resectable without undue risk to the patient. This includes partially resected tumors with measurable tumor left behind. It does not include grossly resected tumors with microscopic disease at the margins or resected tumors with preoperative/intraoperative rupture. Lymph node involvement is considered to constitute stage III disease and may require evaluation with a second laparotomy after the initial four courses of chemotherapy.
Stage IV tumors are those which present with measurable metastatic disease to lungs or other organs.

From Vos A. Primary liver tumors in children. Eur J Surg Oncol 1995;21:101–105.

**TABLE 29-3. PEDIATRIC ONCOLOGY GROUP STAGING OF HEPATOBLASTOMA**

An alternative staging system is that developed by the International Society of Pediatric Oncology based on the number of liver segments involved as determined by preoperative imaging studies.[135] This pretreatment classification scheme (PRETEXT) is shown in Figure 29-5 and is useful in determining resectability preoperatively.[136] In this system, the liver is divided into four sectors—namely, an anterior and a posterior sector on the right, and a medial and a lateral sector on the left. Staging groups are assigned according to tumor extension within the liver as well as the presence or absence of involvement of the hepatic vein, portal vein, regional lymph nodes, or distant metastases. The classification overlaps considerably but is not synonymous with the more conventional POG staging system, which is based on postsurgical tumor status. This classification scheme has been shown to correlate well with resectability and ultimate survival in a large European study.[136] The current U.S. Intergroup Study stratifies patients according the postsurgical staging system but will also attempt to validate the PRETEXT staging system with respect to tumor resectability and outcome.[137]

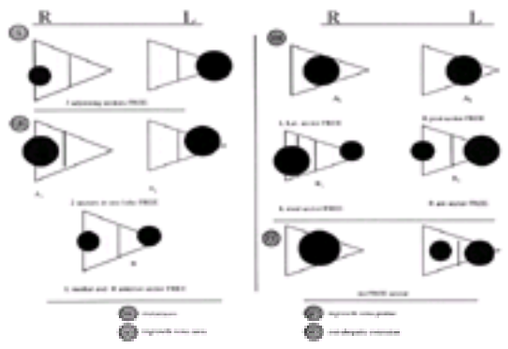

**FIGURE 29-5.** International Society of Pediatric Oncology PRETEXT staging scheme according to surgical anatomy. The liver is divided into four sectors. The number of sectors free is related to stage as follows: PRETEXT I, three adjoining sectors free (tumor only in one sector); PRETEXT II, two adjoining sectors free (tumor involving two sectors); PRETEX III, one sector or two nonadjoining sectors free (tumor involves two or three sectors); PRETEXT IV, no free tumor (tumor in all four sectors). E, extrahepatic direct spread to hilar lymph nodes; M, distant metastases; P, portal vein involvement; V, hepatic vein involvement.

## PATHOLOGY

The usual gross presentation of a primary hepatic neoplasm, whether benign or malignant, is an expansile solitary mass ( Fig. 29-6, Fig. 29-7A, and Fig. 29-8A). The exceptions are some hemangiomas and hemangioendotheliomas, rare hepatoblastomas, adenomas, and hepatocarcinomas with multiple nodules, and in a few infants, diffuse hepatomegaly secondary to systemic dissemination of megakaryoblastic leukemia or neuroblastoma. Encapsulation is limited to some adenomas, although pseudocapsules secondary to compressive atrophy of the adjacent parenchyma can be deceptive with respect to an operative margin. Spontaneous focal necrosis of the rapidly growing hepatoblastoma is common (Fig. 29-6), and many of them have large telangiectatic vessels, both of which account for highly diverse images and Doppler patterns and may lead to erroneous diagnosis, especially in infants when the serum AFP level does not reflect the expected values (i.e., poorly differentiated small cell hepatoblastomas may not have elevations, whereas benign hemangioendotheliomas and mesenchymal hamartomas sometimes can be associated with significant excesses).

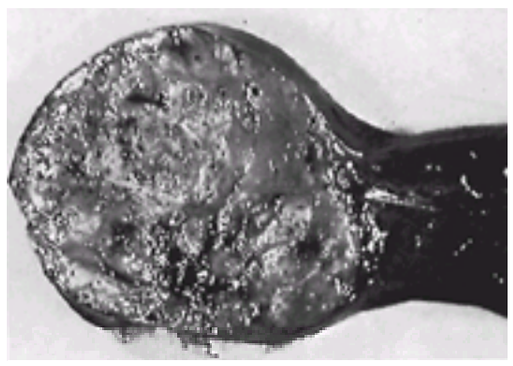

**FIGURE 29-6.** Gross specimen showing hepatoblastoma. The large tumor mass expands the liver. The cut surface is slightly variegated in appearance; one area is hemorrhagic.

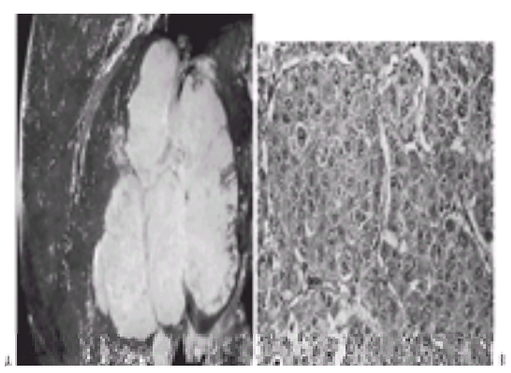

**FIGURE 29-7. A:** Large primary liver cell carcinoma in a noncirrhotic liver. **B:** A well-differentiated hepatocellular carcinoma. Note large polygonal hepatic cells with central nuclei. Mitoses are plentiful.

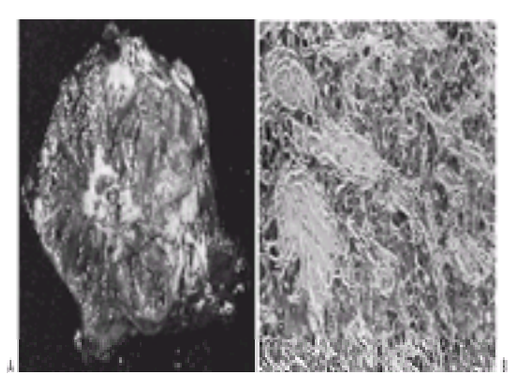

**FIGURE 29-8. A:** Gross specimen showing fibrolamellar carcinoma. Large tumor mass is multilobulated and centrally scarred. **B:** The large polygonal hepatic cells

that make up this tumor are separated by almost acellular collagen. This is the classic appearance of the fibrolamellar variant of hepatocellular carcinoma.

Hepatoblastomas arise from a precursor to the mature hepatocyte and therefore display many histologic patterns, ranging from undifferentiated small cells that coexpress cytokeratin and vimentin reflecting neither epithelial nor stromal differentiation ( Fig. 29-9)[138] to embryonal epithelial cells resembling the histology of the liver at 6 to 8 weeks of gestation (Fig. 29-10) to well-differentiated fetal hepatocytes virtually indistinguishable in cytologic and architectural growth pattern from the normal fetal liver (Fig. 29-11). Rarely, the entire tumor is composed of only one cell type; 5% of the 189 cases reviewed for the POG from 1986 to 1995 were small cell undifferentiated, whereas 7% were pure well-differentiated fetal with minimal mitotic activity ( Fig. 29-11A). The remainder were mixtures of diverse epithelial cells in varying proportions and of cells intermediate among the broad categories, with either discrete nodules of a single cell type or intimate intermingling of diverse cytologies (Fig. 29-9 and Fig. 29-12). Well-differentiated fetal cells having significant mitotic activity (greater than 2 in 10 high-power microscopic fields) ( Fig. 29-11B) very rarely occurred in “pure” form except in small biopsies of stages III and IV cases. Twenty-one percent were classified as “mixed” hepatoblastoma because of stromal derivatives, particularly osteoid-like protein deposits, occasional rhabdomyoblasts, and even more rarely, chondroid elements ( Fig. 29-12).

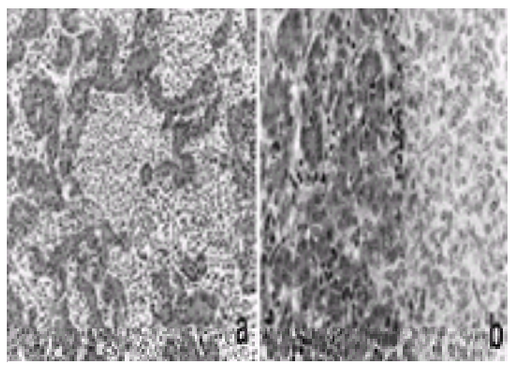


**FIGURE 29-9.** The small undifferentiated type of hepatoblastoma can be thoroughly intermingled with epithelial tubules, as in **(A)**, or form discrete nodules, as in **(B)**. These are the cells that have sometimes failed to respond to conventional chemotherapy and recurred to produce fatalities. Such foci may be readily missed in a small biopsy or fine needle aspirate.

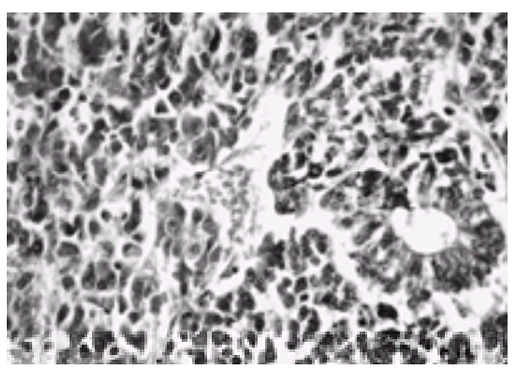

**FIGURE 29-10.** Embryonal hepatoblastoma. Primitive tubules formed by small epithelial cells with minimal cytoplasm are very similar to the liver of 6- to 8-week embryos. Less cohesive cells of the same type are also present. Such foci are mitotically active and often aneuploid.

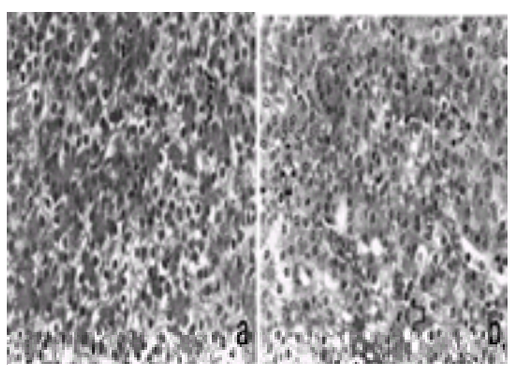


**FIGURE 29-11.** Well-differentiated fetal hepatoblastoma. Both images demonstrate cords of uniform neoplastic hepatocytes smaller than normal cells of the fetal liver with a higher nuclear to cytoplasmic ratio. In **(B)**, arrows point to two mitotic figures in part of 1 high-power field. Only patients with completely resected tumors, as in **(A)**, with few or no mitoses, are classified as having “favorable” histology and spared chemotherapy in current protocols.

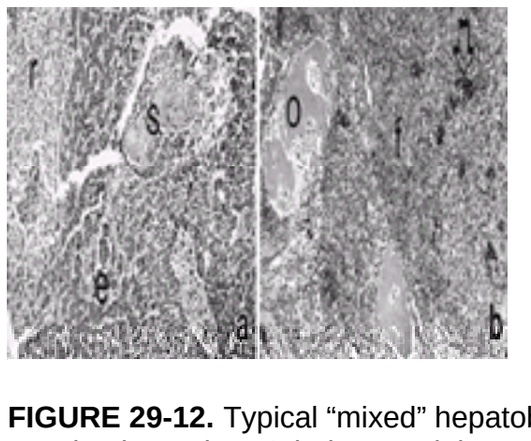


**FIGURE 29-12.** Typical “mixed” hepatoblastoma with diverse histologic features. In **(A)**, the epithelium is relatively primitive, with small cells having minimal cytoplasm growing in cords or tubules, reminiscent of the embryonic liver (e). A nest of keratinizing squamous epithelium is present (s), as well as a zone of elongated spindle cells having the cross-striations of rhabdomyoblasts (r). In **(B)**, the epithelium is more mature “fetal” in type (f) and contains several clusters of hematopoietic cells (*arrow*) as in the normal fetal liver. The mesenchymal component resembles osteoid (o).

Five percent of the hepatoblastomas were designated “teratoid” because a few cells reflecting neural or neural crest origin were present. [139] These included glial cells, neurons, and melanocytes. True teratomas arising in the liver of infants have a full range of tissues, including brain-like and extraembryonal derivatives such as

yolk-sac and trophoblastic cells.[140]

Rarely, enteroendocrine derivatives containing chromogranin and diverse hormones, such as gastrin, serotonin, or somatostatin, are found in mixed hepatoblastomas and even more rarely as pure primary hepatic tumors.[141] Small squamous pearls are frequent in mixed and teratoid tumors (Fig. 29-12A). Very rarely, glandular or ductal forms resembling the embryonal intestine or fetal bile ducts, respectively, were found. The rare rhabdoid tumor arising in the liver shares with small undifferentiated cells the coexpression of epithelial and mesenchymal intermediate filaments. Such cells may occur rarely as a small part of an otherwise typical hepatoblastoma[140] in which their influence on prognosis is unknown, but when they occur in young infants and comprise the entire tumor they suggest a very poor prognosis.[142,143] Although the name *rhabdoid* suggests a relationship to skeletal muscle, these tumors do not contain muscle proteins. True rhabdomyoblasts may be components of hepatoblastomas (Fig. 29-12A), and there are full-fledged primary embryonal rhabdomyosarcomas of the liver that arise in close association to biliary epithelium, often having a polypoid growth pattern and causing obstructive jaundice. [144,145]

When hepatoblastoma cells with either fetal or embryonal cytology grow in trabeculae of 20 to 100 or more cells rather than in the 2- to 4-cell thick cords of the fetal liver, the pattern is called *macrotrabecular,* as suggested by Gonzalez-Crussi and colleagues.[146] Eighteen percent of our series contained macrotrabecular foci and one-third of them were stage IV at presentation. This aggressivity may reflect the fact that such histology can be indistinguishable from hepatocarcinoma when it occurs in pure form (Fig. 29-1 and Fig. 29-13).

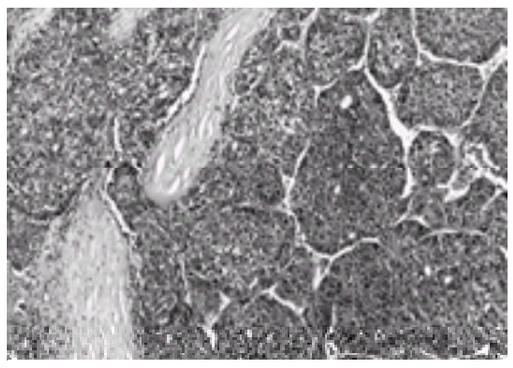

**FIGURE 29-13.** Macrotrabecular hepatoblastoma. Such foci may be indistinguishable from hepatocarcinoma (compare to Fig. 29-1B).

Hepatocarcinomas in children do not differ histologically from those in adults ( Fig. 29-7B), although the fibrolamellar variant (Fig. 29-8B) is more common in adolescents and younger adults. Fibrolamellar carcinomas arise in otherwise normal livers, unlike most hepatocarcinomas, and for that reason are more readily resected and in most series have a higher rate of cure.

A few biliary tract carcinomas have occurred in older children with antecedent cystic or inflammatory disease. [147] They are very resistant to treatment. Solitary benign bile duct cysts are amenable to surgical excision. They may be mature forms of infantile mesenchymal hamartoma.[2]

Angiosarcomas rarely arise de novo and have been reported in children as young as 4 and 5 years following removal of benign hemangiomatous hamartomas in infancy[2] or simultaneously in livers containing hemangiomas. [148] These highly infiltrative lesions disseminate widely within the liver and metastasize to lungs and lymph nodes. The benign hemangioendotheliomas may be solitary masses or multiple in the liver or part of a syndrome of angiomas in many organs and even the placenta of an infant. High-output congestive heart failure is more common in the latter circumstance than with solitary lesions. [149]

Undifferentiated, or embryonal sarcomas, may also present in middle-aged children subsequent to or contemporaneous with a benign infantile mass lesion, a mesenchymal hamartoma.[150,151 and 152] Most arise de novo. These sarcomas generally present as large expansile masses but infiltrate the adjacent parenchyma widely and spread via veins through the liver and to the lungs. Despite the name *undifferentiated*, many of them contain diverse elements displaying maturation of mesenchymal derivatives, including vessels, pericytes, smooth muscle, lipomatous, and fibrohistiocytic features in addition to large bizarre cells with irregular processes and multiple cytoplasmic globular inclusions containing secretory proteins such as alpha $_1$-antitrypsin. The putative pathogenetic relationship between infantile mesenchymal hamartomas, which are typically single expansile masses composed of cystic spaces lined by either biliary epithelium or endothelial cells in a loose myxoid stroma, and the sarcomas is inexplicable by cytogenetic analyses, which thus far have not shown related abnormalities (see the section Acquired Genetic Changes in Tumors).

One other hamartoma of the liver is the angiomyolipoma, a feature of tuberous sclerosis. Although this is most often a tumor of adult females in whom the differential diagnosis from hepatocarcinoma on imaging and in fine-needle aspirates has been the subject of most reports, there have been a few examples in children, not all of whom have had confirmed tuberous sclerosis. The antibody to homatropine methylbromide, which stains the adipocytes and myoblasts of the hamartoma but not hepatocytes, has proved useful in the examination of fine-needle aspirates. One case of malignant transformation has been reported recently. [153]

Another benign tumor occurring in older children and adolescents that may prove problematic on imaging and in fine-needle aspiration cytology is the hepatocellular adenoma. It may be difficult to distinguish from well-differentiated carcinoma. A few cases of carcinoma arising in young adults with glycogen storage disease 1a have been especially confusing because even when these patients have multiple tumors, most are benign. [154] The benign tumors are well reviewed in Patterson.[155]

The relative infrequency of liver tumors in children means that few centers care for more than one or two malignant examples each year. Therefore, it is not surprising that 10.6% of 123 biopsy cases submitted for pathology review in a recent U.S. Intergroup study were incorrectly interpreted. Among the errors were neuroblastoma, Wilms' tumor, and choriocarcinoma.[140]

## TREATMENT

Despite the small number of new cases of hepatoblastoma yearly, cooperative group trials have enabled the development of treatment protocols for hepatoblastomas, resulting in a dramatic increase in survival, particularly for initially unresectable tumors. These trials are discussed in more detail below. HCCs in childhood are sufficiently rare that it has not been feasible to develop separate protocols. Therefore, HCCs have been treated either according to standards in adult centers or similarly to hepatoblastomas, albeit with less success. The approach to the less common malignant tumors of the liver such as rhabdomyosarcoma, other sarcoma variants, and malignant rhabdoid tumors has in general paralleled the approach to tumors of similar histology at other sites, and the rarity of these tumors has precluded the development of treatment protocols. As orthotopic liver transplantation has become a more commonly available and successful option for hepatoblastoma and HCC, allowing definitive surgical resection, it may be that patients with unresectable liver tumors of the less common histologic types may also benefit from transplantation (discussed in the section Liver Transplantation).[156]

### Surgery

Surgical resection is the most important therapeutic modality in treatment of liver tumors. Features that limit surgical resection are involvement of both lobes, involvement of the porta hepatis, or bulky lymphadenopathy. Assessment is usually made by review of CT or magnetic resonance imaging, sometimes with the aid of hepatic arteriogram. The PRETEXT staging system based on the number of liver sectors involved is helpful in determining resectability, together with the extent of extrahepatic extension, involvement of vena cava or portal vein, and the presence of metastatic lesions. Resectability is ultimately determined at the discretion of the surgeon. Surgical resection usually involves hepatic lobectomy or trisegmentectomy. Lymph nodes at the porta hepatis should be sampled, as they represent the primary site of lymphatic drainage. Nodes in the celiac or paraaortic regions should be examined and sampled if suspiciously enlarged. If possible, pulmonary lesions should be surgically resected, as this is thought to improve survival. [157,158 and 159] Although as described below, chemotherapy regimens have facilitated the conversion of unresectable to resectable tumors, in all studies done to date, highest survival rates are observed in tumors in which disease is resected initially. Therefore an

attempt should be made to resect primary as well as metastatic disease.

Resection is curative for most of the benign liver tumors, including the hepatic adenoma, the mesenchymal hamartoma, and hemangioendothelioma. Treatment of hemangioendothelioma is controversial, as surgical resection of large lesions has substantial risks such that alternative strategies are often used, including shrinkage with arterial embolization, corticosteroids, interferon, or cyclophosphamide with variable degrees of success. [149,160] In a review of 30 cases of mesenchymal hamartoma, surgical excision was the only treatment used, with no recurrences observed,[161] although occasionally these tumors show late recurrence or malignant transformation.[162]

**Chemotherapy**

In the 1960s and 1970s, it became evident that hepatoblastoma was a chemosensitive tumor, but early survival rates were low, in the range of 20% to 30%. Agents shown to be effective included vincristine, 5-fluorouracil, and doxorubicin. [163,164 and 165]

Early studies from the Children's Cancer Study Group and the POG documented beneficial effects of adjuvant chemotherapy, consisting of sequential doses of vincristine, doxorubicin, cyclophosphamide, and 5-fluorouracil in completely resected malignant liver tumors. For surgically resected patients, only 1 in 16 (6%) recurred when given this adjuvant regimen compared to 7 of 11 (64%) recurrences in a previous group not given adjuvant chemotherapy. In patients with unresectable hepatic tumors, survival was very poor in those given vincristine, act, and cyclophosphamide. However, when 5-fluorouracil was given in addition to those three agents, the response rate was 44% (12 of 27), although only 7 of 27 (26%) had a sustained response of 20 months or more. [166]

The introduction of cisplatin into chemotherapy regimens for hepatoblastoma markedly improved survival in unresectable tumors. When given as a single agent, a response was shown in four of five patients in a series from St. Jude Children's Hospital. [167] In a subsequent POG pilot study, when cisplatin was added to a regimen with vincristine and 5-fluorouracil, 9 of 11 patients with initially unresectable hepatoblastoma had a complete or partial remission. Five of these patients had measurable pulmonary disease and four achieved a complete disappearance of pulmonary lesions. The average interval of disease control following cisplatin was three times that of doxorubicin.[168]

The survival rate was further improved in a subsequent trial of continuous infusion cisplatin and doxorubicin given over 4 days. [169] The 2-year survival on this regimen was reported as 66%. Douglass and coworkers[170] reported in a POG study that children with resectable hepatoblastoma treated with cisplatin, vincristine, and 5-fluorouracil had a survival rate of 90%. This high survival rate excluded patients with completely resected tumors that were of pure fetal histology, who were cured without adjuvant therapy. Of patients with initially unresected tumors treated with the same regimen for four courses, 24 of 31 patients (77%) became completely resectable. The survival rate at 4 years of 67% was comparable to that observed with cisplatin and doxorubicin given as continuous infusion. Only one of eight patients with metastatic disease survived on this regimen. Two of the 33 patients developed doxorubicin cardiomyopathy, and because of this, subsequent protocols have favored cisplatin in combination with vincristine and 5-fluorouracil rather than in combination with doxorubicin. Doxorubicin, however, remains to be considered an effective agent for refractory or recurrent tumors. The Children's Cancer Group observed similar outcomes for initially unresectable tumors using continuous-infusion doxorubicin and cisplatinum.[169]

In Europe, several multiinstitutional studies have contributed to the treatment of hepatoblastoma. The International Society of Pediatric Oncology Epithelial Liver Group (SIOPEL) reported presurgical treatment of all hepatoblastoma patients, resectable and unresectable, with cisplatin and doxorubicin given by continuous infusion. The overall survival of all patients at 5 years was 75%. [171] The German Cooperative Pediatric Liver Tumor Study HB-89 treated initially unresectable tumors with ifosfamide, cisplatinum, and doxorubicin with response allowing subsequent resection in 76% of patients. [172] A summary of the most recent published chemotherapy regimens from the United States and Europe is shown in Table 29-4.

| Study Group | Scheme[a] | Overall survival (length of follow-up) | N | Reference |
|---|---|---|---|---|
| Children's Cancer Group | Cisplatin, 100 mg/m² / Doxorubicin, 20 mg/m², day 1-4 } 4 courses | 67%/ (2 yr) | 35 | 169 |
| Pediatric Oncology Group | Cisplatin, 90 mg/m² (course 1), followed by Cisplatin, 90 mg/m² / Vincristine, 1.5 mg/m² / 5-fluorouracil, 600 mg/m² } courses 2-5 | 67%/ (4 yr) | 60 | 170 |
| International Society of Pediatric Oncology | Cisplatin, 80 mg/m² over 24 h / Doxorubicin, 60 mg/m² over 48 h } 4 courses → surgery → 2 courses | 75%/ (5 yr) | 154 | 171 |
| German Society of Pediatric Oncology and Hematology | Ifosfamide, 3.5 g/m² / Cisplatin, 100 mg/m² / Doxorubicin, 60 mg/m² } 2 or 4 courses → resection → 2 courses | 35%/ (median 64 mo) | 72 | 172 |

[a]Reader should consult references for full schema.
[b]Includes only patients with unresectable disease at presentation.
[c]Includes patients with all stages at presentation.

**TABLE 29-4. RECENT CHEMOTHERAPY REGIMENS FOR HEPATOBLASTOMA**

A present intergroup study in the United States is examining the possibility of using amifostine to reduce the toxicity of cisplatin-based regimens. Because of the historically high survival rates in children with completely resected hepatoblastomas of well-differentiated pure fetal histology with minimal mitotic activity, this subgroup is being observed without use of adjuvant chemotherapy. The poor responsiveness to current protocols of hepatoblastomas in infants having pure or predominantly small undifferentiated histology indicates the need for additional agents. The Children's Oncology Group is exploring the use of irinotecan and liposomal doxorubicin in recurrent hepatoblastoma. Despite aggressive chemotherapy, 25% to 30% of initially unresectable tumors remain resistant to treatment.

In the cases in which surgical resection is impossible, other approaches have been tried with only moderate success. The introduction of chemotherapy via the hepatic artery has been tried with some success in children. [173,174 and 175] Embolization with an absorbable gelatin sponge (Gelfoam) has also been tried, as has ligation of the hepatic artery.[176,177,178,179 and 180] Targeting with specific antibodies against AFP has also been tried on an experimental basis. [177]

Chemotherapy for HCC has been much less successful than that for hepatoblastoma. Complete resection is the basis for survival, but unfortunately only 10% to 20% of HCCs are resectable. When children with HCC were treated with cisplatinum and doxorubicin in a Children's Cancer Study Group regimen identical to that used successfully in hepatoblastoma, only 2 of 14 patients survived. European studies with different regimens have not demonstrated significantly different overall survival. In the German Cooperative Study HB-89, only 3 of 10 patients with unresectable HCC treated with ifosfamide, cisplatinum, and doxorubicin had a favorable outcome.[172]

The fibrolamellar variant of HCC tends to have a more favorable outcome, presumably because it is more often resectable at diagnosis, and the remainder of the liver is normal.[8,10] Lack and colleagues[8] report an average survival of 28.5 months in patients with the fibrolamellar variant of HCC compared to 4.2 months in other HCCs. The fibrolamellar variants tend to present at a lower stage. However, there was no significant difference from other types of HCC in combined studies of POG and Children's Cancer Study Group in which 28 patients with HCC (14 with fibrolamellar and 14 nonfibrolamellar HCC) were compared stage for stage. [181]

Among the rarer histologic types of liver cancers it is more difficult to reach a definitive treatment recommendation. A response to ifosfamide, carboplatin, and etoposide has been reported in an undifferentiated sarcoma of the liver. [156] Angiosarcoma of the liver has been successfully treated with multi-agent chemotherapy in some but not all cases.[181,183]

**Radiation**

Radiation therapy historically has not played a major role in the treatment of hepatoblastoma. A survey of European institutions indicated that in some cases radiation was helpful in controlling gross or microscopic residual disease. In two of eight inoperable tumors, control was achieved by use of radiation. However, toxicity reported included radiation hepatitis, scoliosis, and bowel obstruction. [184] Radiation has not been extensively used in cooperative group trials in the United States except as an

adjunct in unresectable tumors that remain unresectable even after chemotherapy.[185]

### Liver Transplantation

Whereas tumor resection historically has implied conventional techniques of subtotal hepatic resection, strides have been made over the last two decades in introducing total hepatectomy and orthotopic liver transplantation as a treatment modality in childhood liver tumors, particularly hepatoblastoma and HCC. Liver cancers now account for approximately 2% of all liver transplants in children.[186] An early series of liver transplantation for malignant disease in the 1960s, 1970s, and early 1980s showed promise for this technique, although tumor recurrences posttransplant were common.[187] Survival has shown to be best in tumors that are unifocal and intrahepatic compared to the multifocal tumors and tumors with extrahepatic spread.[188] In most centers, however, extrahepatic spread limits the possibility of liver transplant.

In a recent series of 31 children with initially unresected liver tumors treated initially with chemotherapy followed by total hepatectomy and liver transplant, the 5-year posttransplantation survival was 83% for hepatoblastoma and 68% for HCC.[189] As newer agents have resulted in less toxicity related to control of graft rejection, the quality of life posttransplantation has improved.[190] Remaining challenges in liver transplantation for liver cancer in children include better defining the role of posttransplant adjuvant chemotherapy and prioritization of candidates as recipients for a limited number of donor livers.

### Prognostic Factors

The most important prognostic factor in malignant liver tumors is complete tumor resection. Unfortunately most hepatoblastomas are not amenable to primary surgical total disease resection. The past ten years of POG accessions revealed 67% high-stage tumors with 41% of new cases of hepatoblastomas presenting as stage III and 26% as stage IV. Similarly, in a previous intergroup study, high-stage tumors represented 70% of new cases.[181] Fortunately, most hepatoblastomas that are initially deemed unresectable can be rendered resectable by initial treatment with combination chemotherapy consisting of cisplatin-, carboplatin-, or doxorubicin-containing regimens.

A feature of liver tumors also known to have important prognostic value is the degree of mitotic activity, which may be measured by routine histologic sections, flow cytometry, or by immunohistochemical stains for DNA synthesis. These observations have been made on hepatoblastoma and HCC but also apply to sarcomas, which tend to be aneuploid and inflammatory myofibroblastoma.

Among hepatoblastomas, the presence of pure fetal histology, which is seen in a small percentage of cases, has prognostic significance in that patients with these tumors have an exceptionally good outcome. Kasai and Watanabe[191] observed early that fetal histology was associated with a favorable outcome. In the series reported by Weinberg and Finegold,[2] six of six patients with surgically excised tumors of pure fetal histology and low mitotic activity were cured without the need for additional chemotherapy in contrast to survival of only 2 of 19 patients with resected tumors of other histologies, suggesting that the fetal histology is a key factor influencing cure rate. To date, every known patient with a pure fetal hepatoblastoma and minimal mitotic count has been cured by primary resection with the only three reported exceptions found on review to have contained small undifferentiated cells that constituted the recurrence.[192]

Among patients with unresected tumors who undergo chemotherapy initially because of unresectability, the reduction in AFP level is a good predictor of outcome. A report from the Children's Cancer Group demonstrated that a decline in serum AFP levels early in the course of chemotherapy is a reliable measure of response and indicates a favorable outcome.[128] Patients whose initial AFP levels decreased by 2 logs in the initial four courses of chemotherapy had a 75% survival, whereas patients whose AFP did not decrease 2 logs before second surgery had a very poor survival.

Studies of DNA content have shown that diploid tumors and tumors with low-proliferation index may have a better prognosis than do aneuploid tumors or tumors with a high proliferative index.[193,194] Recent reports of comparative genomic hybridization suggested that addition of material on chromosome 8q or chromosome 20 conferred a poorer prognosis in a small series.[84] Prospective studies examining the role of chromosomal trisomies of 2, 8, or 20 in predicting outcome are in progress.

## FUTURE DIRECTIONS

As toxicity from antirejection therapy improves for liver transplantation, this may become more of a treatment of choice to be considered in patients with otherwise unresectable tumors. In addition, as the need for cadaveric livers for transplantation exceeds the supply, the use of living-related donors who undergo partial hepatectomy with transplantation of a lobe into young infants is becoming recognized as a feasible and sometime more desirable option than cadaveric donors.[195]

Hepatoblastoma is a tumor in which identification of the high-risk individual may facilitate early detection and enhance the ability to cure with limited therapy. A recent series of infants with fumarylacetoacetate hydrolase deficiency (tyrosinemia type 1) known to be at very high risk for HCC underwent treatment with NTBC [2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione], an inhibitor of an enzyme upstream in the catabolic pathway. Both the liver failure and incidence of carcinoma diminished, although some children developed carcinomas despite early and continuous treatment.[196] Efforts to control this and other metabolic conditions associated with cirrhosis will contribute to possibly decreasing the development of hepatic tumors associated with antecedent liver damage.

The recognition of individuals predisposed to hepatic malignancies, whether because of known genetic syndromes such as Beckwith-Wiedemann syndrome or FAP, or because of a history of prematurity and perhaps other pertinent exposures, viral hepatitis, or chronic cholestatic conditions known to be associated with cirrhosis or other factors, sets the stage for development of surveillance strategies. Efforts toward early detection should be made in the high-risk individual to increase the probability of initial surgical resection, which will increase the likelihood of cure while lessening treatment-associated morbidity.

An additional challenge is the need for introduction of novel therapies for both hepatic carcinomas and the rarer hepatic tumors such as the undifferentiated small cell variant or tumors with rhabdoid histology, which have been refractory to conventional chemotherapy.

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# RENAL TUMORS

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## INTRODUCTION

Wilms' tumor is the most common primary malignant renal tumor of childhood and is the paradigm for multimodal treatment of a pediatric malignant solid tumor. Developments in surgical techniques and postoperative care, recognition of the sensitivity of Wilms' tumor to irradiation and several active chemotherapeutic agents have led to a dramatic change in the prognosis for most patients with this once uniformly lethal malignancy. This chapter reviews the epidemiology, molecular biology, pathology, treatment, and prognosis of children with Wilms' tumor, emphasizing the central role of randomized clinical trials in the progress that has been achieved.

## EPIDEMIOLOGY

From 1975 to 1995, the annual incidence of Wilms' tumor in the United States was 7.6 cases per million children younger than 15 years. [1] Wilms' tumor represented 6% of childhood cancers and the total incidence in the United States was estimated at 500 cases per year. The incidence rate is slightly higher for black populations but substantially lower in Asians, both nationally and internationally. [2] Wilms' tumor in the United States is slightly less frequent in boys than in girls. The male to female ratio is 0.92:1.00 for those with unilateral disease and 0.60:1.00 for those with bilateral disease. [3] The tumor presents at an earlier age among boys, with the mean age at diagnosis for those with unilateral disease being 41.5 months compared to 46.9 months among girls. The mean age at diagnosis for those who present with bilateral disease is 29.5 months for boys and 32.6 months for girls. [3]

In a few children, Wilms' tumor occurs as part of a recognized congenital malformation syndrome ( Table 30-1). The *W*ilms' tumor, *a*niridia, *g*enitourinary malformation, mental *r*etardation (WAGR) syndrome results from a germline deletion at chromosome arm 11p. [4,5] Children with pseudohermaphroditism, degenerative renal disease (glomerulonephritis or nephrotic syndrome) and Wilms' tumor have Denys-Drash syndrome, [6,7] which is associated with mutations within the same 11p chromosomal segment. [8] Hemihypertrophy may occur as an isolated abnormality or as a component of the Beckwith-Wiedemann syndrome (BWS), which includes macroglossia, omphalocele, and visceromegaly. [9,10] Wilms' tumor has been reported in patients with other overgrowth syndromes, including the Perlman [11,12] and Simpson-Golabi-Behmel syndromes. [13] Although cases of Wilms' tumor have also been reported in patients with neurofibromatosis, [14] Sotos' syndrome, [15] and Klippel-Trénauny-Weber syndrome, [16] these associations are weaker and not clearly causal. [17] Wilms' tumor has been described in patients with genetic instability syndromes, including the Bloom syndrome [18] and incontinentia pigmenti [19] and has been observed in families affected by Li-Fraumeni syndrome. [20] The causal significance of these latter observations is also unclear.

| Anomaly or syndrome | Number of patients | Prevalence rate (per 1,000) |
|---|---|---|
| Wilms' tumor, aniridia, genitourinary malformation, mental retardation syndrome | 52 | 7.5 |
| Denys-Drash syndrome | 28 | 4.0 |
| Beckwith-Wiedemann syndrome | 74 | 10.7 |
| Sporadic hemihypertrophy | 171 | 25.1 |
| Cryptorchidism (boys only) | 49 | 46.6 |
| Hypospadias (boys only) | 64 | 20.0 |

**TABLE 30-1. CONGENITAL ANOMALIES AND SYNDROMES IN 6,890 PATIENTS REGISTERED BY THE NATIONAL WILMS' TUMOR STUDY GROUP FROM 1980 TO 1999**

Stimulated by an early report of an excess of Wilms' tumor among children whose fathers worked in occupations having the potential for contact with hydrocarbons or lead,[21] a series of epidemiologic studies has investigated the potential role of parental occupational and environmental exposures as risk factors for Wilms' tumor.[22,23,24,25,26,27,28 and 29] These were mostly small case-control studies that had serious methodological weaknesses, including inaccurate exposure assessment and unavoidable bias in the selection of the control series. Although several studies noted an excess of fathers who worked as machinists or vehicle mechanics, no consistent positive findings have emerged. Previously reported associations between maternal smoking, tea consumption, and hypertension during pregnancy likewise have not been confirmed. On balance, the fact that incidence patterns vary more with ethnicity than geography combined with the inconsistent findings from case-control studies suggest that genetic risk factors are likely to be of greater consequence for Wilms' tumor than are environmental risk factors.

## GENETICS AND MOLECULAR BIOLOGY

Wilms' tumor appears to result primarily from the loss of function of certain tumor suppressor genes as opposed to the activation of oncogenes. Based on a statistical analysis of the age of onset of unilateral and bilateral tumors, Knudson [30] and Knudson and Strong[31] proposed in the early 1970s that Wilms' tumors develop as a consequence of two rate-limiting mutational events. The first event can be prezygotic (i.e., a constitutional or germline event) or postzygotic. If the first event is prezygotic, the tumor is potentially hereditary, and affected individuals are at risk of developing multiple tumors because all their cells are affected by the first event. Consequently, only one new event in any one cell is required for tumor development. By contrast, the nonhereditary or sporadic form results from two relatively rare independent, somatic mutations in a single cell. Because it is unlikely for these two events to occur independently in more than one cell, patients with nonhereditary Wilms' tumor are unlikely to develop more than one tumor. The clearest example of the two-event theory is the inactivation of both copies of *RB1* in the development of retinoblastoma. In the 1990s, however, it became clear that the genetic pathways leading to the development of Wilms' tumor are more complicated. [32] Several chromosomal regions have been associated with Wilms' tumor, including band 11p13, which harbors the Wilms' tumor suppressor gene *WT1*; band 11p15, the location of the putative Wilms' tumor gene *WT2*; chromosome arm 17q, which harbors the familial locus *FWT1*; chromosome arm 19q, the location of *FWT2*; and chromosome arms 16q, 1p, 7p, and 17p, the location of *p53*. The first four of these loci have been implicated in the actual development of Wilms' tumor. The remaining four may not predispose to Wilms' tumor but may be associated with aspects of the phenotype, including outcome of treatment.

### *WT1*

The first genetic region involved in the development of Wilms' tumor was revealed by the study of patients with the WAGR syndrome. Children with the WAGR syndrome were shown to have heterozygous germline deletions at band 11p13.[5] This constitutional deletion was thought to represent the first hit and provided the initial clue to the location of a gene involved in the development of Wilms' tumor. Molecular analysis of this specific region led to the identification of *WT1*.[33,34 and 35] It is now known that the WAGR deletion encompasses a number of contiguous genes, including the aniridia gene *PAX6*[36] and the Wilms' tumor suppressor gene *WT1*, so the syndrome constitutes a contiguous gene syndrome.

Characterization of *WT1* revealed ten exons encoding a protein with a predicted molecular weight of 45 to 49 kd, depending on the presence or absence of two alternative splicing events. Cells expressing *WT1* produce four distinct messenger RNAs (mRNAs), reflecting the presence of the two alternative splice sites. The most prevalent *WT1* mRNA species is the one with both alternative splices present.[37] The carboxyl terminus of the WT1 protein contains four zinc finger domains of the histidine-cysteine type, a motif that in other proteins is associated with sequence-specific binding to DNA. The WT1 amino terminus contains a domain rich in proline and glutamine, a motif characteristic of transcriptional factors.[33,34] This suggests that WT1 functions as a regulator of the transcription of other genes.[38] The identity of one of the genes targeted by the WT1 protein during normal kidney development, amphiregulin,[39] suggests a role of WT1 as a transcriptional regulator during kidney differentiation. Although several genes appear to be regulated by *WT1* in different *in vitro* models, the WT1-regulated pathway involved in tumorigenesis remains unclear.

*WT1* was established as a Wilms' tumor suppressor gene by several criteria. First, tumor-specific homozygous deletions of all or part of *WT1* were described in several Wilms' tumor cases.[34,40] Second, certain patients with bilateral Wilms' tumor were found to have constitutional *WT1* mutations.[41] Third, investigators reported homozygous somatic mutations in patients with nonhereditary unilateral Wilms' tumor.[42,43 and 44] Thus it is clear that *WT1* is a tumor suppressor gene involved in the development of certain Wilms' tumors. Because approximately 30% to 40% of Wilms' tumors manifest loss of heterozygosity (LOH) for the region encompassing *WT1*, a similar incidence of tumors with underlying *WT1* mutations was expected. Surprisingly, though, the incidence of *WT1* mutations remains at less than 10%,[45] despite the identification of a potential mutational hot spot involving the first exon of the gene.[46]

*WT1* was initially thought to be a classic tumor suppressor gene, requiring the loss of both alleles for tumor development. It has now become clear that specific alterations to only one allele may also contribute to abnormal cell growth. Patients with Denys-Drash syndrome harbor constitutional point mutations in only one *WT1* allele,[8,42] resulting in a dominant negative oncogene.[47] The abnormal protein product is thought to disrupt the function of the normal gene product (from the remaining normal allele) through the formation of protein complexes or through abnormal interactions with DNA targets. Thus, loss of *WT1* function may result from a dysfunctional mutation in only one allele (a dominant effect). The phenotypic effects of the constitutional *WT1* mutations found in Denys-Drash syndrome are in fact far more severe than those resulting from complete deletion of *WT1*, seen in patients with the WAGR syndrome. This supports the concept that the altered WT1 protein in Denys-Drash syndrome is dysfunctional rather than nonfunctional. Most Denys-Drash mutations are single–base-pair mutations, with a mutational hot spot in exon 9.[8] Potential dominant negative *WT1* mutations have also been seen in sporadic Wilms' tumor specimens containing only one mutated *WT1* allele.[48,49 and 50]

The characterization of *WT1* has provided insight not only into the mechanisms underlying the development of Wilms' tumor but also into those involved in genitourinary development.[51,52 and 53] Study of the embryonic-lethal homozygous *WT1*-knockout mouse reveals failure of kidney development and that WT1 expression is necessary for the survival and differentiation of the metanephric blastema.[52] These mice also exhibit lack of gonadal development, which may reflect lack of interaction between *WT1* and sex-determining genes such as *SRY*, Müllerian-inhibiting substance, and the androgen receptor.[53]

The unexpectedly low frequency of *WT1* mutations suggests that other genetic alterations account for the majority of Wilms' tumors. Given the complex pattern of *WT1* transcription, DNA-binding specificity, and transcriptional regulating capacity, however, it remains possible that *WT1* may be involved in more cases than are immediately obvious or that a further Wilms' tumor suppressor locus may reside in the 11p13 region.

### *WT2*

The existence of a second Wilms' tumor gene on chromosome arm 11p was first appreciated by the fact that a subset of Wilms' tumors undergoes LOH for markers telomeric to 11p13, not including *WT1*. This second locus, designated *WT2*, may explain the association of Wilms' tumor with BWS,[54,55] a congenital overgrowth syndrome characterized by overgrowth—ranging from gigantism to regional hypertrophy (hemihypertrophy) or visceromegaly and macroglossia, hyperinsulinemic hypoglycemia and a predisposition to several embryonal neoplasms. Although most BWS cases are sporadic, approximately 15% are familial or associated with chromosomal abnormalities.[56] Linkage analysis has suggested that the locus for the familial form of BWS maps to band 11p15.[54,55] Whether the *BWS* gene and *WT2*

are one and the same gene is unclear. Interestingly, in tumors with LOH, it is invariably the maternal copy of band 11p15 that is lost, [57,58 and 59] suggesting that the two copies of *WT2* are not functionally equivalent. This phenomenon is thought to be due to genomic imprinting, a process whereby one allele is marked, or imprinted, in a parental-specific manner to be functionally inactive. The imprint is not coded for by the DNA sequence because it must be reversible during gametogenesis.

Thus far at least three candidate imprinted genes have been proposed for *WT2*, including the insulin-like growth factor II gene (*IGFII*),[60,61 and 62] *H19* (an untranslated RNA),[63,64] and *p57*$^{kip2}$.[65,66 and 67] *IGFII* encodes an embryonal growth factor that is highly expressed in fetal kidney and Wilms' tumors. [60] In humans only the paternal *IGFII* allele is expressed,[61,62] and increased expression of this imprinted gene could conceivably contribute to the development of Wilms' tumor and BWS. Because LOH for 11p15 markers in Wilms' tumors represents loss of the inactive maternal allele with duplication of the active paternal allele, the LOH may cause up-regulation of the “oncogene” *IGFII* rather than loss of a tumor suppressor gene. Consistent with this hypothesis is the observation that some Wilms' tumors that have not lost heterozygosity at 11p15 have instead a relaxation (or loss) of the imprint at the *IGFII* locus.[61,62] This results in biallelic and presumably increased expression of the *IGFII* gene. Similarly, constitutional loss of the imprint has been associated with some cases of BWS. [68]

There is also evidence for the involvement of an adjacent locus *H19*. *H19* is imprinted in a reciprocal manner to *IGFII*, with expression restricted to the maternal allele.[63] Introduction of *H19* into Wilms' tumor and rhabdomyosarcoma cell lines suppresses both growth and tumorigenicity, suggesting that loss of *H19* may contribute to the development of Wilms' tumor. [64] Transfection of a subchromosomal 11p15 fragment not including *IGFII* or *H19* into a rhabdomyosarcoma cell line, however, also results in growth suppression, [69] suggesting additional or alternate tumor suppressor genes at 11p15. Notably, loss of the maternal *H19* allele and loss of the imprint both result in inactivation of *H19*.[62,63] Finally, *p57*$^{kip2}$, a paternally imprinted gene encoding a cell cycle regulator and located 500 kb centromeric to *IGFII*, might also be involved in Wilms' tumorigenesis. Although expression of the *p57*$^{kip2}$ gene is reduced in some Wilms' tumors,[66,67,70] the relationship between decreased expression and Wilms' tumor development remains to be determined.

In summary, there is strong evidence that alterations at one or more imprinted loci on band 11p15 underlie the development of BWS and may predispose to the development of Wilms' tumor. Whether BWS and Wilms' tumor result from alterations to the same gene or whether multiple loci are affected by a single genetic mechanism remains uncertain.

### Other Possible Wilms' Tumor Loci

LOH for markers on the distal long arm of chromosome 16 has been found in approximately 20% of Wilms' tumors, [59,71] and loss of the short arm of chromosome 1 has been found in approximately 10% of cases.[72] Both events may be associated with adverse outcome,[72] so the putative underlying tumor suppressor loci are likely involved in tumor progression rather than initiation. One of the major objectives of the ongoing fifth National Wilms' Tumor Study (NWTS-5) is to confirm the prognostic significance of 1p and 16q LOH and to ascertain their clinical significance within each tumor stage.

Analyses of karyotypes from Wilms' tumors and from patients with Wilms' tumor have identified recurrent deletions and translocations involving the short arm of chromosome 7.[73,74,75 and 76] In addition, tumor-specific LOH for 7p markers has been detected in 14% of 35 Wilms' tumors analyzed, [77] together suggesting the presence of a genetic locus involved in the genesis of Wilms' tumor at chromosome arm 7p. Clinical correlates of 7p LOH have not been published, so the exact role of this possible Wilms' locus, if any, has yet to be determined.

Alterations of the *p53* tumor suppressor gene and its encoded protein are the most frequently encountered genetic events in human cancer, having been reported in almost every type of sporadic neoplasm.[78] Moreover, constitutional *p53* mutations are associated with the multi-cancer predisposition syndrome named after Li and Fraumeni.[79] Based on a study of the family histories of 176 children with Wilms' tumor, [20] the suggestion was made that Wilms' tumor might be a rare component of the Li-Fraumeni syndrome. Given the rarity of the Li-Fraumeni syndrome and the relatively higher frequency of Wilms' tumor, however, this observation may represent a sampling bias rather than a true association. Meanwhile, the incidence of *p53* mutations in sporadic Wilms' tumors, unlike that observed in many other malignancies, is relatively low, probably less than 10%.[80,81 and 82] Results of one study suggest that *p53* alterations in Wilms' tumor are associated with anaplastic histology, [80] a histopathologic variant of Wilms' tumor with poor prognosis. A second study, [81] however, identified a *p53* mutation in a favorable histology Wilms' tumor that carries an excellent prognosis. Further studies are required to ascertain the exact biologic significance of *p53* mutations in Wilms' tumor.

### Familial Wilms' Tumor

Familial Wilms' tumor accounts for 1% to 2% of all cases.[83,84 and 85] In view of the absence of parental consanguinity in such families, the mode of inheritance is generally thought to be autosomal dominant, with variable penetrance and expressivity. [86] Only one-tenth of the kindreds reported involve affected parents. [85] More often, the disease occurs in siblings, cousins, or other relatives. The fraction of Wilms' tumor cases that are of hereditary origin was originally estimated to be 38%, but this has since been revised downward to between 15% and 20%. [87] If the two-stage model is correct, one would expect to see increasing numbers of Wilms' tumors in the offspring of survivors of the disease, especially if they had the familial, bilateral, or other “hereditary” form. However, a survey of 191 children of 99 patients with unilateral Wilms' tumor did not identify a single case of cancer. [88,89] True estimates of the risks in offspring of Wilms' tumor patients will await follow-up of offspring from survivors of the National Wilms' Tumor Study Group (NWTSG) and other large patient populations.

Linkage analyses in several pedigrees have excluded both *WT1* and *WT2* on chromosome arm 11p from conferring susceptibility to Wilms' tumor.[90,91] Similarly, chromosome arm 16q has been excluded as the site for the familial Wilms' tumor locus. [92] Several pedigrees have now demonstrated linkage to band 17q12-21 and the locus termed *FWT1*. Neither familial nor sporadic Wilms' tumors have undergone LOH for this region, as would be expected if *FWT1* were a tumor suppressor locus.[93] A second locus, *FWT2*, has been mapped to chromosome region 19q13.3-q13.4.[94] As some families exhibit evidence against linkage to WT1, FWT1, and FWT2,[94,95 and 96] however, the existence of yet additional Wilms' tumor loci must be assumed. Comparative genomic hybridization analysis of familial tumors has implicated chromosome arms 4q, 9p, 20p, and 3q as areas of potential interest. [97] These regions have not previously been implicated in the biology of Wilms' tumor and may potentially harbor genes predisposing to familial Wilms' tumor or involved in sporadic Wilms' tumor.

In summary, the development and progression of Wilms' tumor involves a number of genetic loci. Ongoing research will undoubtedly help uncover which genetic and epigenetic (imprinting) alterations are truly important in the biology of this tumor. This knowledge in turn will facilitate a better understanding of the disease as well as provide new prognostic factors useful for determining the intensity of therapy.

To this same end, measures of telomerase levels and function have been studied in a relapsed case:cohort series of Wilms' tumors. Virtually all tumors demonstrated evidence of telomerase expression or function, consistent with other malignant neoplasms. One measure, mRNA expression of *hTERT*, the RNA component that encodes the catalytic component of telomerase, was found to be associated with risk of recurrence in univariate analyses. [98] Further studies are necessary to confirm this finding and to discern its clinical utility.

## PATHOLOGY

The earliest known specimen of Wilms' tumor is on display in the Hunterian Museum of the Royal College of Surgeons in London, England. [99] This specimen of bilateral renal tumors from a young infant was prepared by John Hunter, who died in 1793, and is in an excellent state of preservation. Subsequently, Rance, Eberth, and others recorded examples of this tumor. A detailed analysis of 145 cases was published in 1897 by Walker, but the monograph by Max Wilms is regarded as the classic description.[100]

Since its classic description by Wilms, nephroblastoma has been noted for its tremendous histologic diversity and ability to display a wide variety of cell and tissue types.[101] Much of the past success of the NWTSG has relied on the accurate subclassification of tumors into high- and low-risk types, allowing the intensity of therapy to be modified appropriately. Pathologic review of these tumors centrally at the NWTSG Pathology Center from 1969 onward has ensured the consistency of pathologic assessments and allowed for this progress. Further subclassification will likely depend on categorization of Wilms' tumors on the basis of molecular features.

### Gross Appearances and Patterns of Extension

Most Wilms' tumors are solitary lesions; however, a substantial number are multifocal, with 7% involving both kidneys either at presentation or subsequently, and an

additional 12% arising multifocally within a single kidney. [102] There is no predilection for either side, and the tumor may arise anywhere within the kidney. Extrarenal Wilms' tumors are rare and generally occur in the retroperitoneum adjacent to but unconnected with the kidney. [103] Others have been found in the pelvis, inguinal region, and thorax and are thought to arise in displaced metanephric elements, mesonephric remnants, or teratomas.

Wilms' tumors commonly have a uniform pale gray or tan color on section, although hemorrhage and necrosis frequently impart a variegated appearance. Cysts are commonly encountered and may be a dominant feature. Not uncommonly, especially in infants, a polypoid extension into the pyelocalyceal lumen may resemble the growth pattern seen in botryoid rhabdomyosarcoma.[104] Tumors with a predominance of differentiating stromal elements may have a firm texture, but most specimens are notably soft and friable. This may contribute to the local spread of tumor cells when a tumor is ruptured either before or during operation. Wilms' tumors are usually sharply demarcated, relatively spherical masses with a “pushing” border relative to the adjacent renal parenchyma. A distinct intrarenal pseudocapsule composed of compressed, atrophic renal tissues commonly surrounds Wilms' tumors. This feature may help to distinguish Wilms' tumor from mesoblastic nephroma, clear cell sarcoma, rhabdoid tumor, and renal lymphoma, all of which demonstrate infiltrative borders. Wilms' tumors not infrequently involve the renal vein and may extend up the inferior vena cava to reach the right atrium.

**Histology**

The most distinctive microscopic feature of Wilms' tumor is its diversity. The classic nephroblastoma is made up of varying proportions of three cell types—blastemal, stromal, and epithelial—often recapitulating various stages of normal renal development ( Fig. 30-1). Blastemal cells are undifferentiated small blue cells that may be arranged in diffuse or organoid patterns. [105] Epithelial structures such as glomeruli and tubules simulating the normal nephrogenic zone are commonly seen. Less commonly, papillary formations or heterologous squamous or mucinous epithelium unlike any in the normal developing kidney are identified. Stromal differentiation is usually manifest as immature spindled cells, heterologous skeletal muscle, cartilage, osteoid, or fat. [101,106] Not all specimens are triphasic, and biphasic as well as monophasic patterns are frequently encountered. The monophasic patterns often present diagnostic difficulties. Monophasic blastemal Wilms' tumor are often highly invasive and may raise the differential diagnosis of other small round blue cell tumors, such as primitive neuroepithelial tumor, neuroblastoma, and lymphoma. Similarly, monophasic undifferentiated stromal Wilms' tumor may simulate primary sarcomas such as clear cell sarcoma of the kidney, congenital mesoblastic nephroma, or synovial sarcoma. Other stromal Wilms' tumors show a predominance of skeletal muscle differentiation varying from well differentiated (rhabdomyomatous) to poorly differentiated skeletal muscle (rhabdomyoblastic). The distinction of a pure rhabdomyoblastic Wilms' tumor (which is quite rare) from a primary renal rhabdomyosarcoma is often impossible on morphologic grounds. Such lesions should be treated on the basis of their line of differentiation rather than their putative cell of origin. Finally, purely tubular and papillary Wilms' tumor may at times be difficult to distinguish from papillary renal cell carcinoma. [105] In fact, it has been suggested that there may be a biologic link between nephroblastic lesions and some renal cell carcinomas. [105]

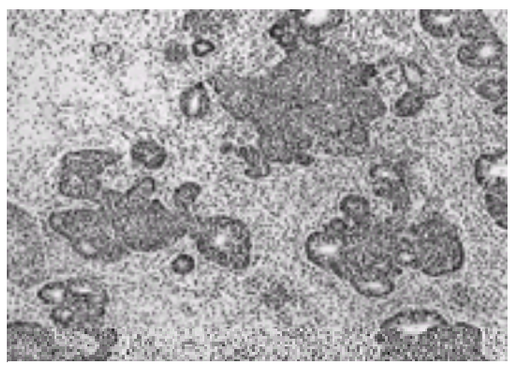

**FIGURE 30-1.** Triphasic Wilms' tumor, with well-defined tubules surrounded by dense clusters of blastemal cells and zones of pale-staining stromal differentiation (hematoxylin and eosin, 100×).

Wilms' tumors often contain scattered cysts; however, not uncommonly tumors present that are predominantly or purely cystic. Those that have grossly identifiable solid nodules of tumor are best classified as cystic Wilms' tumors. Tumors that are devoid of any solid nodular growth but which contain immature nephrogenic elements within their septa are designated *cystic partially differentiated nephroblastoma* (CPDN). Others contain only mature cell types and are classified as cystic nephroma (CN). CPDN and CN are both curable by surgery alone and are thought to represent the most favorable end of the Wilms' spectrum in children. Either lesion can recur if ruptured or incompletely excised, so distinction of CN from CPDN is of little clinical significance. [107] A familial association between CN and the often cystic pleuropulmonary blastoma has been reported.[108,109]

A correlation between the histologic pattern and the clinical behavior of Wilms' tumor has long been sought. The most significant correlation that has been reported is the distinction of “favorable” from “unfavorable” histology Wilms' tumor. When anaplastic nuclear changes, as described below, are not present, the histology is termed *favorable* because of the generally good outcome for these patients. [101] Other more limited correlations between behavior and histology have been reported. Blastemal-rich tumors tend to be extremely invasive and present at a high stage, but often respond well to chemotherapy. In contrast, predominantly epithelial and rhabdomyomatous Wilms' tumors more frequently present at a low stage, reflecting less aggressiveness, yet are often resistant to chemotherapy. Although these prognostic trends are intriguing, they have not been statistically significant possibly due to the small number of stage IV predominantly epithelial Wilms' tumor available for analysis.[110]

***Anaplastic Wilms' Tumor***

*Anaplasia* is defined by the presence of markedly enlarged polyploid nuclei within the tumor sample ( Fig. 30-2). The criteria for the diagnosis of anaplasia include the following: (a) the identification of nuclei with a diameter at least three times those of adjacent cells; (b) hyperchromasia of the enlarged cells, providing evidence for increased chromatin content; and (c) the presence of multipolar or otherwise recognizably polyploid mitotic figures. [111] All of these features must be identified for the diagnosis of anaplasia, although occasionally, when only a small biopsy is available, the presence of a single multipolar mitotic figure or an unequivocally gigantic tumor cell nucleus will suffice to establish the diagnosis. The frequency of anaplasia is approximately 5% and correlates with patient age. It is rare in the first 2 years of life (2% of NWTS-3 patients) and then increases to a relatively stable rate of approximately 13% in patients older than 5 years. It is significantly more frequent in African-American than in white patients.[111]

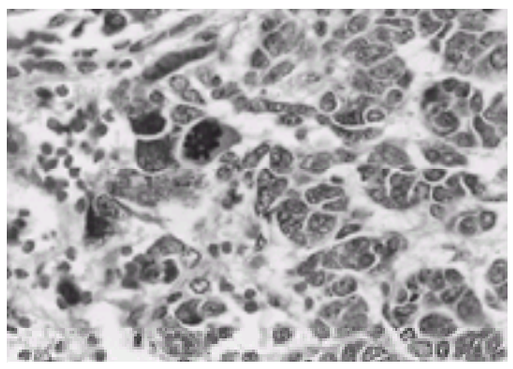

**FIGURE 30-2.** Anaplastic Wilms' tumor with a large, darkly stained multipolar mitotic figure near the center and a markedly enlarged interphase nucleus to its left. Nuclei throughout this field exhibit increased variation in size and shape (hematoxylin and eosin, 600×).

Stage I anaplastic Wilms' tumor has the same excellent prognosis as stage I favorable histology Wilms' tumor, whereas stages II through IV anaplastic Wilms' tumor have a markedly diminished prognosis, suggesting that anaplasia is a marker of resistance to chemotherapy but not of increased aggressiveness or tendency to disseminate.[112] This recognition has resulted in a distinction between tumors showing anaplastic changes that are focal and those that are diffuse. [113] The diagnosis of focal anaplasia requires that cells with anaplastic nuclear changes be confined to sharply circumscribed regions within the primary tumor, and that these cells are not present in any site outside the kidney parenchyma. The diagnostic criteria for diffuse anaplasia includes any one of the following: (a) presence of anaplasia in any extrarenal site, including vessels of the renal sinus, extracapsular infiltrates, or nodal or distant metastases; (b) presence of anaplasia in a random biopsy specimen; (c) unequivocal anaplasia in one region of the tumor, coupled with extreme nuclear pleomorphism approaching the criteria of anaplasia (extreme nuclear unrest) elsewhere in the lesion; (d) presence of anaplasia in more than one tumor slide, unless (i) it is known that every slide showing anaplasia came from the same region of the tumor, or (ii) anaplastic foci on the various slides are minute and surrounded on all sides by nonanaplastic tumor. The distinction between focal and diffuse anaplasia has been demonstrated to be prognostically significant. [113]

### *Nephrogenic Rests*

The existence of precursor lesions to Wilms' tumor has been recognized for many years. [114,115] These nephrogenic rests are found in almost 1% of unselected pediatric autopsies, in 35% of kidneys with unilateral Wilms' tumor, and in nearly 100% of kidneys with bilateral Wilms' tumor. [116] They are composed of abnormally persistent embryonal nephroblastic tissue with small clusters of blastemal cells, tubules, or stromal cells ( Fig. 30-3). Nephrogenic rests are classified by their position within the kidney. Intralobar nephrogenic rests are randomly distributed but tend to be situated deep within the renal lobe, likely reflecting an earlier developmental insult to the kidney. These lesions are commonly stroma rich and intermingle with the adjacent renal parenchyma. Perilobar nephrogenic rests are located at the periphery, are usually subcortical and sharply demarcated, and contain predominantly blastema and tubules. These presumably reflect later developmental disturbances in nephrogenesis. Morphologic distinction of the two types of rests is of interest because they have different clinical and pathologic associations ( Table 30-2).[117] The term *nephroblastomatosis* is used to refer to the presence of multiple nephrogenic rests. Diffuse overgrowth of perilobar nephrogenic rests may produce a thick “rind” of blastemal or tubular cells that enlarge the kidney but preserve its original shape. There are several possible fates for nephrogenic rests. Only a small number develop a clonal transformation into Wilms' tumor. When this happens, the Wilms' tumor is typically spherical and develops a pseudocapsule separating it from the nephrogenic rest. Some nephrogenic rests may become hyperplastic, with dramatic enlargement that preserves the shape of the preceding rest. Such lesions may be histologically indistinguishable from Wilms' tumor unless the interface between the rest and the adjacent normal kidney is present within the sample. Hyperplastic nephrogenic rests may completely regress or differentiate after the administration of chemotherapy. The majority of nephrogenic rests becomes dormant or involutes spontaneously. The presence of nephrogenic rests within a kidney resected for a Wilms' tumor indicates the need for monitoring the contralateral kidney for tumor development, particularly in infants.[118]

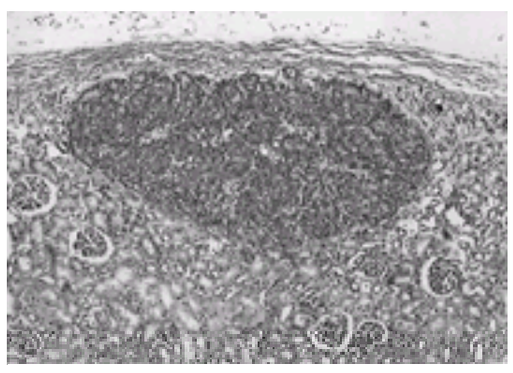

**FIGURE 30-3.** Perilobar nephrogenic rest. An ovoid subcapsular mass of embryonal blastemal and tubular cells is sharply demarcated from the adjacent renal cortex. This was found in the grossly normal portion of the kidney in a 3-year-old child with unicentric Wilms' tumor (hematoxylin and eosin, 125×).

| | Intralobar | Perilobar |
|---|---|---|
| Associated syndromes | Wilms' tumor, aniridia, genitourinary malformation, mental retardation, Denys-Drash | Beckwith-Wiedemann |
| Location within renal lobe | Random, often central | Peripheral |
| Interface with kidney | Intermingling | Distinct |
| Dominant histologic component | Stroma | Blastema or tubules |
| Number | Usually single | Often multiple |

**TABLE 30-2. NEPHROGENIC RESTS: PERILOBAR VERSUS INTRALOBAR**

### Clear Cell Sarcoma of the Kidney

Clear cell sarcoma of the kidney is the second most common pediatric renal neoplasm and is a tumor associated with a significantly higher rate of relapse and death than favorable histology Wilms' tumor. This variant was described in detail in 1978 by three independent groups. [101,119,120] The descriptive term *clear cell sarcoma of the kidney* is based on the staining characteristics of the predominant cell type; others have referred to *bone-metastasizing renal tumor of childhood* because bone is a common metastatic site. Clear cell sarcoma of the kidney has a far wider distribution of metastases than favorable histology Wilms' tumor, both by age and by site. Both spread to the lungs most frequently, but clear cell sarcoma of the kidney has a strikingly increased number of brain, bone, and soft tissue metastases, as well as an extended time period during which metastases may present. [121,122 and 123]

Most clear cell sarcoma of the kidney specimens have a distinct histologic appearance, but a number of variant patterns, such as epithelioid, spindling, myxoid, and cystic, invite confusion with Wilms' tumor or other tumor types. [123,124,125 and 126] As a result, clear cell sarcoma of the kidney remains the pediatric renal tumor most frequently misdiagnosed. The classic morphologic pattern of clear cell sarcoma is biphasic, composed of nests of plump cord cells containing abundant extracellular matrix separated by a network of capillary vascular arcades that are often surrounded by more spindled septal cells ( Fig. 30-4). Finally, clear cell sarcoma can show anaplastic nuclear features identical to those of anaplastic Wilms' tumor. Immunohistochemical studies are useful in the exclusion of other tumors, but no positive diagnostically useful immunohistochemical or genetic markers have been identified to date. [123]

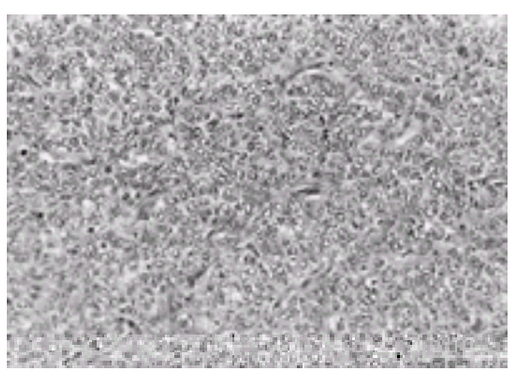

**FIGURE 30-4.** Clear cell sarcoma of the kidney, classic pattern. Cords and nests of pale-stained tumor cells are separated by a delicate but distinct network of fine vascular septa. Nuclei are vesicular, with poorly stained chromatin and inconspicuous nucleoli (hematoxylin and eosin, 200×).

### Rhabdoid Tumor of the Kidney

A distinctive and highly malignant tumor, rhabdoid tumor of the kidney was identified in 1978 by NWTSG pathologists. [101] Rhabdoid tumor of the kidney occurs more frequently in infants, with 85% of cases occurring within the first 2 years of life and a sharp decline thereafter. The diagnosis should be considered highly suspect over the age of 5 years.[127] The tumor is commonly widely metastatic at presentation, and in both NWTSG and Societe Internationale d'Oncology Pediatrique (SIOP) studies, more than 80% of children died within 1 year of diagnosis. [127,128]

Rhabdoid tumors are usually bulky masses centered in the renal hilum, with a grossly indistinct tumor border reflecting aggressive invasion. Prominent intrarenal vascular invasion leads to frequent satellite nodules that may be seen grossly. The tumor characteristically grows as sheets of monotonous discohesive cells characterized by vesicular nuclei, prominent macronucleoli, and hyaline cytoplasmic inclusions ( Fig. 30-5). However, these cytologic features may be variably present and diligent search may be required before diagnostic foci are encountered. In addition, a large number of variant patterns have been described, including sclerosing, epithelioid, spindled, vascular, and lymphomatoid, all of which can simulate other neoplasms. [129] Rhabdoid tumor received its name because of the prominent acidophilic cytoplasm, resembling rhabdomyoblasts. However, it has been amply demonstrated that rhabdoid tumors are not related to myogenic cells and are negative for ultrastructural or other markers of skeletal muscle. The cell of origin for this distinctive tumor remains unknown. [129,130] and [131] It is not a variant of Wilms' tumor and has not been encountered as a focal change in a conventional Wilms' tumor.

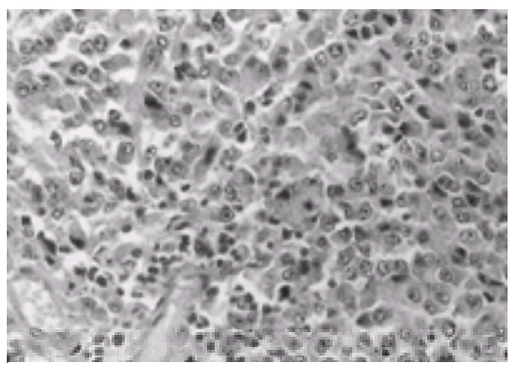

**FIGURE 30-5.** Rhabdoid tumor. Most nuclei have a large, single nucleolus, imparting an “owl's eye” appearance to the nucleus. Several cells, including one near the center, have hyaline globular cytoplasmic inclusions. Ultrastructurally, the latter inclusions consist of whorled masses of intermediate filaments, usually composed of vimentin (hematoxylin and eosin, 600×).

Rhabdoid tumors characteristically show a polyphenotypic immunohistochemical staining pattern, with focal strong positivity for a variety of markers, including cytokeratin, epithelial membrane antigen, desmin, and neurofilament. The presence of scattered clusters of intensely epithelial membrane antigen or cytokeratin positive cells in a background of negative staining is a characteristic of rhabdoid tumors that is unique among the pediatric tumors in the differential diagnosis.

The pathologic diagnosis of renal rhabdoid tumors is complicated by two features. The first is that a variety of renal and nonrenal tumors can show rhabdoid features.[132,133] The majority of such tumors in patients older than 5 years represent other neoplasms. Many of these “pseudorhabdoid” lesions have been identified by immunohistochemistry or other techniques to be carcinomas, melanomas, histiocytic tumors, or sarcomas. The recent establishment of consistent deletion of 22q11-12 in both renal and extrarenal rhabdoid tumors in infants supports the existence of a biologically distinct primary rhabdoid tumor of early childhood. [134,135,136] and [137] The common area of deletion has been recently been mapped to the *hSNF5/INI1* gene.[138] Tumor-specific genetic abnormalities that have been reported include deletions and truncating mutations. Germline mutations have been demonstrated in some children. [139]

A second complicating feature of renal rhabdoid tumors is the association with brain tumors, most of which are located in the midline cerebellum. [140] Many of these tumors have been classified as medulloblastoma or primitive neuroectodermal tumors, although tumors classified as gliomas and ependymomas have been reported. More recently, it has been recognized that infantile rhabdoid tumors of the brain, also known as atypical teratoid tumors, often have the same histologic appearance as undifferentiated medulloblastomas or primitive neuroectodermal tumors yet show the chromosome 22 abnormalities seen in renal rhabdoid tumors. [141] It may be difficult, if not impossible, in individual cases to determine if synchronous or metachronous renal and central nervous system lesions represents metastases or two primary sites in a patient with a predisposition of tumor development.

### Congenital Mesoblastic Nephroma

The term *congenital mesoblastic nephroma* was applied by Bolande and colleagues [142] in 1967 to a distinctive renal neoplasm of infancy. Congenital mesoblastic nephroma occurs predominantly in infants, with a median age of 2 months.[143] Three histologic subtypes have been defined: the classic type (24% of cases), the more frequent cellular type (66% of cases), and mixed type (10% of cases) showing both classic and cellular patterns. [144] The classic subtype of congenital mesoblastic nephroma grows as intersecting fascicles of bland spindle cells with tapered nuclei and pink cytoplasm and is histologically similar to infantile fibromatosis. Mitoses are usually infrequent and necrosis is absent. The cellular subtype of congenital mesoblastic nephroma has a solid, cellular, sheetlike growth pattern of oval or round cells with little cytoplasm and frequent mitoses and necrosis. The mixed type of congenital mesoblastic nephroma features areas resembling both classic and cellular morphologies. Although classic congenital mesoblastic nephromas histologically resemble infantile fibromatosis, the cellular congenital mesoblastic nephroma resembles infantile fibrosarcoma. Recently, a genetic linkage between infantile fibrosarcoma and cellular congenital mesoblastic nephroma was established when the chromosome translocation t(12;15)(p13;q25), initially discovered in infantile fibrosarcoma, [145] was also identified in cellular congenital mesoblastic nephroma. [146,147] and [148] The cloning of the resulting gene fusion has allowed the development of molecular detection assays for this subtype of congenital mesoblastic nephroma. [149] The absence of the fusion product in classic congenital mesoblastic nephroma correlates with its demonstrated absence in infantile fibromatosis.

The most significant clinical and pathologic feature of congenital mesoblastic nephromas is their tendency to grow into the hilar and perirenal soft tissue, often in a subtle fashion. As a result, recurrence or metastasis is seen in up to 20% of patients. [150,151,152] and [153] Because of this tendency, congenital mesoblastic nephroma deserves a radical surgical approach, with efforts to secure a wide margin of uninvolved tissue on all aspects of the specimen, but particularly the medial aspect. After surgery, any residual tumor may recur with astonishing rapidity; therefore, close radiographic follow-up is indicated for the first year. The poor response shown by congenital mesoblastic nephromas to chemotherapy and radiation therapy highlights the importance of surgical excision.

### Renal Cell Carcinoma

The classic forms of renal cell carcinoma of adulthood are quite rare in children in the absence of a genetic predisposition. [154,155] However, there is a growing appreciation that a distinctive, but rare, subtype of renal cell carcinoma presents in adolescence and young adulthood. These tumors are genetically unique in that they have chromosome translocations involving a common breakpoint at Xp11.2.[156,157,158] and [159] These tumors show a distinct male predominance. Histologically, these may show a great resemblance to clear cell renal cell carcinoma, with copious clear cytoplasm. However, one clue to their diagnosis is that they often have a papillary architecture. The genetic alterations commonly associated with papillary renal cell (trisomy 7,17) and clear cell renal cell (3p deletion) carcinomas in adults have not been found in the tumors containing Xp11 alterations. The NWTSG pathology center has encountered other translocations as the sole genetic abnormality in tumors classified as renal cell carcinomas ( *unpublished data*). The clinical significance of these translocations, including the Xp11 translocation, is currently unknown.

Because these tumors are genetically distinct from those found in adults, however, the utility of nuclear grading is questioned.

Another unique and rare renal carcinoma that predominantly involves children is the recently described renal medullary carcinoma. [160] This highly lethal tumor is virtually restricted to patients with sickle cell hemoglobinopathy, most commonly sickle cell trait. This tumor may present in children as young as 3 years and has been reported into middle age; however, the median age at presentation is 13 years. Renal medullary carcinomas are histologically characterized by a rhabdoid phenotype, abundant acute inflammation, a highly invasive growth pattern, and early metastasis. Sickled cells are usually present in the tissue sections and should make the diagnosis evident.

## CLINICAL PRESENTATION

Osler[161] provided the first North American description of the signs and symptoms of the tumor that Wilms described 20 years later. Most children with Wilms' tumor are brought to medical attention because of abdominal swelling or the presence of an abdominal mass. [100] Often this is noticed by a parent while bathing or dressing the child. Abdominal pain, gross hematuria, and fever are other frequent findings at diagnosis. [100] Hypertension, present in approximately 25% of cases, has been attributed to an increase in renin activity. [162]

A characteristic presentation for a subset of Wilms' tumor patients includes rapid abdominal enlargement, anemia, hypertension, and, sometimes, fever. These symptoms may be associated with egg-shell–type calcification visible on plain radiographs of the abdomen. The syndrome appears to be related to sudden subcapsular hemorrhage.[162]

During the physical examination, it is important to note the location and size of the abdominal mass. A Wilms' tumor is usually a large flank mass that does not particularly move with respiration. These findings help differentiate Wilms' tumor from splenomegaly and neuroblastoma, the latter often arising in the celiac axis or extending across the midline because of lymph node involvement. A varicocele secondary to obstruction of the spermatic vein may be associated with the presence of a tumor thrombus in the renal vein or inferior vena cava. Persistence of the varicocele when the child is supine is highly suggestive of venous obstruction. It is also important to specifically note any signs of the Wilms' tumor–associated syndromes marked by the presence of aniridia, BWS-associated facial dysmorphisms, partial or complete hemihypertrophy, and genitourinary abnormalities including hypospadias, cryptorchidism, and pseudohermaphrodism.

### Differential Diagnosis

The chief differential diagnosis for Wilms' tumor is another embryonal tumor, neuroblastoma. The distinction is usually relatively easy, because most neuroblastomas arise from the adrenal gland or the paravertebral sympathetic ganglia, whereas Wilms' tumors are almost always intrarenal. Benign processes and other malignant intrarenal neoplasms can produce clinical and radiographic findings similar to those of Wilms' tumor, however, and as many as 9% of tumors are misdiagnosed without the benefit of biopsy (Table 30-3).[163,164]

| Diagnosis | National Wilms' Tumor Study Group | Société Internationale d'Oncologie Pédiatrique | Total |
|---|---|---|---|
| Neuroblastoma | 9 | 19 | 28 |
| Mesoblastic nephroma | 10 | 4 | 14 |
| Renal cyst | — | 11 | 11 |
| Renal cell carcinoma | 1 | 5 | 6 |
| Polycystic kidney | 5 | — | 5 |
| Multicystic kidney | 3 | — | 3 |
| Hydronephrosis | — | 3 | 3 |
| Hypernephroma | 2 | — | 2 |
| Extrarenal fibrosarcoma | 1 | 1 | 2 |
| Renal carbuncle | 1 | — | 1 |
| Benign teratoma | 1 | — | 1 |
| Adrenal hemorrhage | 1 | — | 1 |
| Renal thrombosis | 1 | — | 1 |
| Renal rhabdomyosarcoma | 1 | — | 1 |
| Renal leiomyosarcoma | 1 | — | 1 |
| Dysplastic kidney | 1 | — | 1 |
| Extrarenal leiomyosarcoma | 1 | — | 1 |
| Multilocular cyst | 1 | — | 1 |
| Lymphangiosarcoma | — | 1 | 1 |
| Incorrect diagnosis | 40/586 (6.8%) | 44/442 (9.9%) | 84/1,028 (8.2%) |

Adapted from D'Angio GJ, Evans AE, Breslow N, et al. The treatment of Wilms' tumor. Cancer 1976;38:633–646; and Lemerle J, Voute PA, Tournade MF, et al. Preoperative versus postoperative radiotherapy, single vein, multiple courses of actinomycin D, in the treatment of Wilms' tumor. Cancer 1975;36:647–654.

**TABLE 30-3. DIAGNOSES IN 1,028 CHILDREN WITH SUSPECTED WILMS' TUMOR**

### Workup

Laboratory evaluation should include a complete peripheral blood count, liver function tests, renal function tests, serum calcium, and urinalysis. Elevation of the serum calcium may occur in children with rhabdoid tumor of the kidney or congenital mesoblastic nephroma. [165,166] Acquired von Willebrand's disease may occur in approximately 8% of patients at diagnosis, and consideration should be given to coagulation assays. [167]

## IMAGING STUDIES

Imaging studies preoperatively and in follow-up should be designed to assist the clinicians managing the child. They must therefore consider the known characteristics of the tumors at presentation and of metastatic patterns based on the tumor type, including favorable and anaplastic histology Wilms' tumor, clear cell sarcoma of the kidney, rhabdoid tumor of the kidney, and congenital mesoblastic nephroma.

Initial imaging studies should be restricted to those necessary to establish the presence of an intrarenal space-occupying lesion, to guide the surgeon, and to assist the radiation therapist should postoperative radiation therapy be indicated. Data of importance to the surgeon include the presence and function of the opposite kidney, whether the opposite kidney is involved with tumor, and the presence and extent of an intravascular tumor thrombus.

The initial radiographic study often selected is an abdominal ultrasound examination ( Fig. 30-6). This will demonstrate whether the abdominal mass is solid or cystic and establish the renal origin of the mass as well as the measurement of the maximum diameter of the mass. Contrast-enhanced computed tomography (CT) of the abdomen (Fig. 30-7) is also recommended to further evaluate the nature and extent of the mass. Although this study may suggest apparent extension of the tumor into adjacent structures such as the liver, spleen, or colon, most children identified as having possible invasion of the liver on CT are found at the time of surgical exploration to have hepatic compression rather than hepatic invasion. [168] In addition the examination may demonstrate small lesions in the opposite kidney that may be nephrogenic rests or Wilms' tumor, although small superficial or intrarenal lesions are frequently not identified even when CT is used. [169] It is important, when CT is used, that the radiologist obtain a supine x-ray film of the abdomen after contrast injection, because this view is necessary for planning and review of radiation therapy.

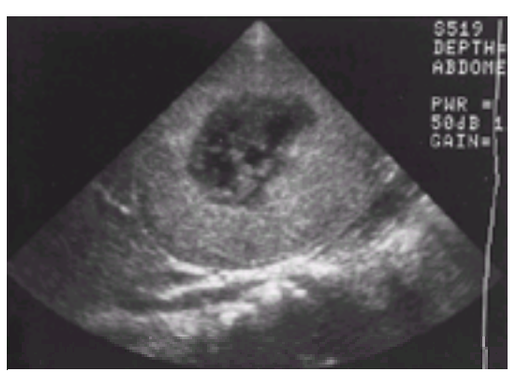


**FIGURE 30-6.** Wilms' tumor sonogram. A prone sagittal scan of right renal fossa demonstrates a large, complex, mainly solid intrarenal mass.

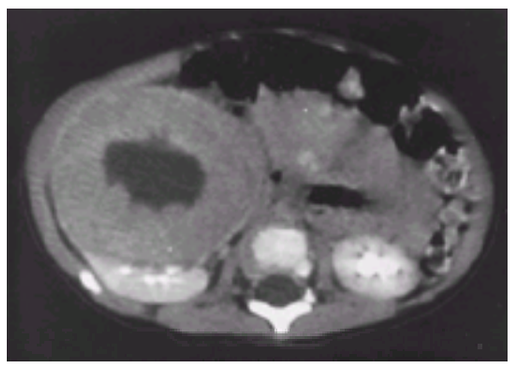

**FIGURE 30-7.** Computed tomography scan of the abdomen showing a right renal tumor and a normal appearing left kidney.

The patency of the inferior vena cava may be demonstrated relatively inexpensively using real-time ultrasonography. When a tumor is identified within that vessel, the proximal extent of the thrombus must be established before operation because extension of the thrombus to the right atrium may result in few if any clinical signs. [170,171] Exceptionally, sudden death has been reported following dislodgement and mobilization of the tumor lying within the draining vessels at laparotomy. [172]

The results of the radiographic studies and real-time ultrasonography provide sufficient information on which to make a decision for laparotomy in most children, although no imaging study unequivocally establishes the histologic diagnosis of Wilms' tumor.

Plain chest radiographs or CT of the chest should be obtained to determine whether pulmonary metastases are present. CT has been compared to conventional radiographic studies in Wilms' tumor patients in two studies. In the first, 9.4% (11 of 117) of the CT scans demonstrated densities not identified on a plain chest radiograph, although none of these patients underwent open lung biopsy for confirmation of the histology of the pulmonary lesions. [173] In the other, only 2.4% (2 of 83) of the CT scans demonstrated densities not identified using *four-view* chest radiography, and one of the lesions was shown pathologically to be a granuloma, not a metastasis.[174] Conversely, another study reported that four of five biopsied solitary lesions from the lungs of children with Wilms' tumor identified only by CT were, in fact, pulmonary metastases.[175] These data suggest that when pulmonary nodules are small enough to be identified by CT only, consideration should be given to biopsying at least one of them.

A radionuclide bone scan and x-ray skeletal survey should be obtained postoperatively in children with clear cell sarcoma of the kidney and other children with pulmonary or hepatic metastases who have suggestive symptomatology. Both studies are necessary because plain radiographs of the bones involved with clear cell sarcoma of the kidney can demonstrate lytic lesions that may not be seen on bone scan.[176,177]

Brain imaging using magnetic resonance imaging or computerized tomography should be obtained on all children with clear cell sarcoma of the kidney or with rhabdoid tumor of the kidney, because both are associated with intracranial metastases. [121,129] Second primary malignant brain tumors of varying histologic type, often arising in the posterior fossa, are also found in patients with rhabdoid tumor of the kidney. [127,129,140]

## STAGING

A single set of criteria is used by the NWTSG to stage all pediatric renal tumors. These criteria have evolved over the years, and the current definitions are listed in Table 30-4. The most significant recent change has been in the criteria for distinguishing between stages I and II. [178] Before NWTS-5, the criteria for stage II included either renal capsular penetration or extension of the tumor past the hilar plane, an imaginary boundary marked by the medial border of the renal sinus. The hilar region, also referred to as the renal sinus, is biologically quite important because it contains the major renal vessels, an important mode of hematogenous and lymphatic spread. The hilar plane lacks a defining anatomic border, however, is often distorted by bulky tumors and can be assessed only on initial gross pathologic examination of the nephrectomy specimen. The hilar plane criterion for staging was removed from NWTS-5 and replaced by the criterion of renal sinus vascular invasion. This definition includes involvement of vessels located in the radial extensions of the sinus into the renal parenchyma but excludes other intrarenal vascular invasion. Applying the new criteria, the difference in survival between stage I and II Wilms' tumors continues to be statistically significant.

| Stage | Description |
|---|---|
| I | Tumor confined to the kidney and completely resected. No penetration of the renal capsule or involvement of renal sinus vessels. |
| II | Tumor extends beyond the kidney but is completely resected (negative margins and lymph nodes). At least one of the following has occurred: (a) penetration of the renal capsule, (b) invasion of the renal sinus vessels, (c) biopsy of tumor before removal, (d) spillage of tumor locally during removal. |
| III | Gross or microscopic residual tumor remains postoperatively, including inoperable tumor, positive surgical margins, tumor spillage involving peritoneal surfaces, regional lymph node metastases, or transected tumor thrombus. |
| IV | Hematogenous metastases or lymph node metastases outside the abdomen (e.g., lung, liver, bone, brain). |
| V | Bilateral renal Wilms' tumors at onset. |

**TABLE 30-4. NATIONAL WILMS' TUMOR STUDY GROUP STAGING SYSTEM FOR RENAL TUMORS**

The most common sites of metastases of Wilms' tumor are the lungs, the regional nodes, and the liver. Of NWTSG patients presenting with hematogenous metastases at diagnosis (stage IV), the lungs were the only site in approximately 80% of cases. The liver, with or without lung involvement, was involved in 15%. [179] Other metastatic sites are distinctly uncommon in Wilms' tumor.

## PROGNOSTIC CONSIDERATIONS

Tumor size, the age of the patient, histology, lymph node metastases, and local features of the tumor, such as capsular or vascular invasion, have in the past been predictive of risk for tumor recurrence or progression. Because prognostic factors are identified retrospectively, however, the significance of previously determined prognostic factors may change when more effective treatment regimens are developed. This in fact has occurred with the sequential evaluation of prognostic factors among children treated on the NWTSG studies.

The histologic finding of anaplasia has been identified as the most important determinant of prognosis. [111,180] More recent analyses have confirmed the importance of histopathology.[113] Likewise, lymph node involvement, as originally suggested by Jereb and colleagues, [181,182] remains an important predictor of treatment failure.

Children entered on NWTS-1 who were younger than 24 months had a significantly better prognosis than did those who were older. The relapse rate was 14.8% for those younger than 2 years compared to 34.7% for those aged between 2 and 4 years and 27.6% for those older than 4 years. [183] The prognostic significance of age and tumor size, however, have lessened as treatment efficacy improved.[183,184] and [185]

Recent research has focused on identification of additional prognostic factors, ideally ones that are independent of stage and histology, that could be used for further

stratification of therapy according to the risk of recurrence.

Possible molecular genetic prognostic factors have been identified in a study of 232 children with Wilms' tumor registered on NWTS-3 and -4. LOH for markers on chromosome arm 16q, which was present in tumor tissue from 17% of those with favorable or anaplastic histology Wilms' tumor, was associated with significantly poorer 2-year relapse-free and overall survival percentages. [72] The difference remained when the analysis was adjusted for stage or histology. LOH for chromosome arm 1p markers, present in tumor tissue from 11% of children with Wilms' tumor, was also associated with poorer relapse-free and overall survival, differences that were not statistically significant however ($p$ = .08 and .12, respectively). By contrast, LOH for 11p markers or duplication of 1q, present in 33% and 25% of cases, respectively, was not associated with any difference in outcome. [72] The possible associations between LOH for chromosome arms 16q and 1p are being tested in NWTS-5.

Several authors have evaluated the relationship between tumor cell DNA content and prognosis in Wilms' tumor. Aneuploidy occurs more frequently in anaplastic histology tumors.[186,187 and 188] Tetraploidy is associated with a worse prognosis in some series, [186,189] and aneuploidy was associated with a worse prognosis among patients with favorable histology Wilms' tumor in one series [190] but not in two others.[188,191] One of the objectives of NWTS-5 study is to define the relationship between cellular DNA content, as determined using flow cytometry, and prognosis.

Based on retrospective studies, measures of nuclear morphometry were proposed to discriminate outcome in Wilms' tumor patients. [192] Further study, however, did not reveal any association between the proposed measures and outcome.[193]

## GENERAL SURGICAL PRINCIPLES

Surgical excision is the initial treatment for most children with Wilms' tumor. The surgeon has an important responsibility to perform safe and complete removal of the Wilms' tumor. Intraoperative events that can have an adverse impact on patient survival include tumor spill, incomplete removal, and surgical complications. Careful removal of the tumor without rupture or spill is mandatory, as these patients have an increased risk of local abdominal relapse. [194] Complete tumor resection improves patient survival. The surgeon's responsibility is not only to remove the primary tumor intact but also to assess the tumor spread precisely. Accurate staging is essential for the subsequent determination of the need for radiation therapy and the administration of the appropriate chemotherapy regimen.

### Technique

The transperitoneal approach to the tumor has been the standard procedure since the work of Ladd and colleagues. [195] The flank approach should never be used, because adequate staging cannot be performed, and the contralateral kidney cannot be examined. [196] Once the peritoneal cavity is entered, thorough exploration of the abdominal cavity is carried out.[181,197] One should assess the liver and regional periaortic lymph nodes and look for other evidence of tumor spread. The presence or absence of lymph node metastases is of major importance in determining treatment and relapse-free survival. [183,184 and 185] Thus, selective sampling of nodes is necessary for accurate staging. There is no evidence that extensive lymph node removal alters the outcome in patients with Wilms' tumor, and thus formal retroperitoneal lymph node dissection is not recommended.

Exploration of the contralateral kidney should be performed before nephrectomy. The colon is reflected and Gerota's fascia opened so that the kidney can be palpated and inspected on all surfaces for evidence of a synchronous bilateral tumor or evidence of nephrogenic rests. These may not be identified on preoperative imaging studies, especially if they are small or flat. Any abnormalities of the opposite kidney should be biopsied. The presence of bilateral Wilms' tumor alters the surgical approach significantly (see below); thus the status of the contralateral kidney must be determined *before* nephrectomy.

After the contralateral exploration is completed, radical nephrectomy is performed. The colon is mobilized off the tumor and reflected medially, preserving the colonic blood supply.[198] Gentle handling of the tumor throughout the procedure is mandatory to avoid tumor spillage, because this complication results in a sixfold increase in local abdominal relapse.[199] Although early ligation of the renal vein does not appear to have an appreciable effect on survival, separate ligation is performed before mobilization of the tumor but only if exposure is adequate. More important, the surgeon should be certain that the contralateral renal vessels, aorta, iliac, or superior mesenteric arteries have not been mistakenly ligated.[198]

Palpation of the renal vein and inferior vena cava should be performed to exclude intravascular tumor extension before vessel ligation. Wilms' tumor extends into the inferior vena cava in approximately 6% of cases and may be clinically asymptomatic in more than 50% of these. [200,201] Involvement of the renal vein or inferior vena cava by tumor does not adversely affect the prognosis if appropriate treatment is given. Identification of intracaval extension on the preoperative imaging studies allows the surgical team to adequately prepare for the operative procedure.

Surgery should not be overlooked as a cause of morbidity in children with Wilms' tumor. A recent review of NWTS-4 patients undergoing primary nephrectomy found an 11% incidence of surgical complications.The most common complications were hemorrhage and small bowel obstruction. Risk factors associated with increased surgical complications are higher local tumor stage, incorrect preoperative diagnosis, intravascular extension, and en bloc resection of other visceral organs. [202] Heroic attempts to excise en bloc all or parts of adjacent organs to which the tumor appears to be adherent are not warranted because such procedures are associated with an increased risk of surgical complications. Wilms' tumors are generally very large, and the gross appearance of the tumor at the time of surgery can be misleading in interpreting tumor extent. These tumors often compress and adhere to adjacent structures without frank invasion, and in the majority of cases, pathological evidence of organ invasion is not present.[202]

### Preoperative Therapy

Prevailing North American practice is to proceed with primary surgery after appropriate workup. This is because treatments can best be modulated in their intensity according to accurate histopathologic and staging criteria, which are obscured when preoperative treatments are given. However, there are occasional patients in whom primary nephrectomy may pose too great a risk.[202] The NWTSG has recommended preoperative chemotherapy for a few select groups of patients, including those with tumor extension into the inferior vena cava above the hepatic veins, [171] those found to be unresectable at surgical exploration, [203] and children with bilateral renal tumors.[196,204] Studies conducted by the SIOP[205,206] and the NWTSG experience have shown that pretreatment with chemotherapy almost always reduces the bulk of the tumor. This makes tumor removal easier and may reduce the frequency of surgical complications. [171] Preoperative chemotherapy does not result in improved survival rates, however, and does result in the loss of important staging information. [207]

All patients with tumors considered to be unresectable after imaging studies should undergo initial exploration to assess operability and to obtain a biopsy of the tumor. It is important to keep in mind that the error rate in the preoperative diagnosis of renal masses after roentgenographic assessment is 5% to 10% ( Table 30-3).[163,164] Percutaneous needle biopsy of lesions presumed to be inoperable is also to be avoided because the histologic diagnosis based on material obtained from a needle biopsy has an error rate of up to 7.4%.[208,209] Patients who are staged by imaging studies alone are at risk for either understaging or overstaging. Thorough exploration of the abdomen is necessary to detect evidence of extrarenal extension of tumor. If suspicious lymph nodes or other metastatic deposits are found, these should be biopsied to document tumor involvement. If the tumor is found to be unresectable, biopsy of the tumor can be followed by chemotherapy or radiation therapy. This will generally produce tumor shrinkage to allow resection. Unresectable tumors should be outlined with titanium clips for possible postoperative radiation therapy. These clips are radiopaque but scatter the beam less than other metal clips during postoperative CT scanning.

Inoperable tumors must be treated as stage III, using vincristine, dactinomycin, and doxorubicin (regimen DD-4A; see Fig. 30-10). If preoperative therapy for inoperable tumors is given based on imaging alone, with or without a needle biopsy, the local tumor should also be considered as stage III. Once there has been an adequate reduction in the size of the tumor to facilitate nephrectomy, definitive resection should be completed. In general, radiographic reevaluation should be performed at week 5 after initiating chemotherapy. The operative procedure can be performed shortly thereafter. Serial imaging evaluation is helpful to assess response, but radiographic evidence of persistent tumor mass can occasionally be misleading. Failure of the tumor to shrink can be due to predominance of skeletal muscle or differentiated elements, and a second look procedure to confirm persistent tumor may be necessary. [203,210]

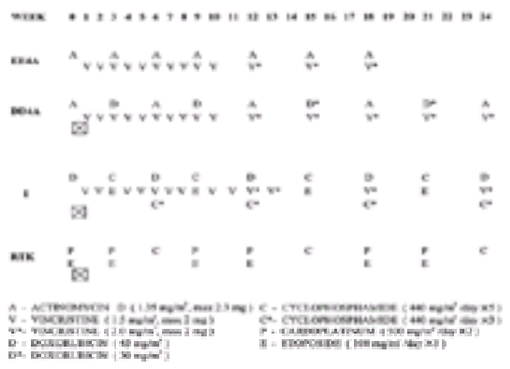

**FIGURE 30-10.** Treatment regimens used on National Wilms' Tumor Study 5.

Patients who do not respond adequately can be considered for preoperative irradiation, which may produce enough shrinkage to permit nephrectomy. The suggested dose is 1,200 to 1,260 cGy, administered using daily fractions of 150 to 180 cGy. Vincristine should be administered weekly during the period of radiation therapy. Postoperative radiation therapy is given to all patients who did not receive it preoperatively. This recommendation is based on the results of the SIOP-6 nephroblastoma trial, in which a significantly higher infradiaphragmatic relapse rate was reported in nonirradiated children given only postoperative chemotherapy.  The radiation therapy field is enlarged beyond the flank if the surgical findings warrant. After radiation therapy, patients should continue on chemotherapy until they have completed the regimen for stage III disease.

Primary surgical excision of the tumor with caval extension is associated with increased surgical morbidity. [201] For vena caval involvement below the level of the hepatic veins, the caval thrombus can be removed via cavotomy after proximal and distal vascular control is obtained. Generally the thrombus will be free floating, but if there is adherence of the thrombus to the caval wall, the thrombus can often be delivered with the passage of a Fogarty or Foley balloon catheter. Patients with extension of intracaval tumor thrombus above the level of the hepatic veins should receive preoperative chemotherapy to shrink the tumor and thrombus. [171,211] This facilitates complete removal of the tumor with decreased morbidity.

In patients with intracardiac extension of tumor that has not responded to preoperative combination chemotherapy, a median sternotomy and midline abdominal incision provide excellent exposure of the right atrium and the intrapericardial portion of the inferior vena cava. [171,212] Extracorporeal circulation is necessary in these situations. This finding does not necessarily adversely affect life expectancy, however, because all 16 NWTSG children reported with intracardiac tumors of favorable histology survived.[171]

**Bilateral Disease**

Various reports record the incidence of synchronous bilateral Wilms' tumor as ranging from 4.4% to 7.0% and that of metachronous bilateral Wilms' tumor from 1.0% to 1.9% of Wilms' tumor patients.[196,213,214] As imaging techniques have improved, a greater percentage of bilateral lesions have been diagnosed preoperatively. In a review of NWTS-2 and -3 patients with bilateral Wilms' tumor, CT of the abdomen was accurate in diagnosing synchronous bilateral Wilms' tumor in 83%. [169] However, a review of 122 patients with synchronous bilateral Wilms' tumor enrolled in NWTS-4 noted that 7% of bilateral lesions were not identified by the preoperative imaging studies.[118] These data emphasize the importance of contralateral renal exploration at the time of laparotomy for apparent unilateral disease.

The historical approach to the surgical management of children with bilateral Wilms' tumors included nephrectomy of the more involved side combined with excisional biopsy or partial nephrectomy of the presumably smaller lesion in the remaining kidney. Consequently, many patients with synchronous bilateral Wilms' tumor developed significant renal insufficiency, some to the extent of requiring renal transplantation. [215] An NWTSG review found that 9.1% of patients with synchronous bilateral Wilms' tumors and 18.8% of those with metachronous bilateral tumors developed renal failure. [216] The most common etiology for renal failure was the need for bilateral nephrectomy for persistent or recurrent tumor in the remaining kidney after initial nephrectomy. Treatment-related injury (radiation-induced damage, surgical complications) to the remaining kidney was the second leading cause of renal insufficiency. Renal insufficiency secondary to hyperfiltration-induced injury (focal glomerulosclerosis) was less common.[216]

In 1977, NWTSG investigators reported 30 children with bilateral Wilms' tumor. [204] Nineteen of the patients had been managed with unilateral nephrectomy with or without contralateral heminephrectomy or biopsy, whereas 11 children had undergone a renal biopsy alone followed by chemotherapy or radiation therapy. Overall survival was excellent (87% 2-year survival) with no obvious difference between the two groups. A subsequent larger series of 145 bilateral Wilms' tumor patients enrolled in NWTS-2 and -3 confirmed the observation that patients undergoing initial biopsy followed by postoperative chemotherapy had an equivalent survival to patients undergoing initial surgical resection (83%). [196,217] Most important, it was noted that nephrectomy could be avoided entirely in almost 50% of the group of patients undergoing initial biopsy.

The management presently recommended, therefore, is initial bilateral renal biopsy with staging of each kidney. Abnormal lymph nodes or other lesions suggestive of extrarenal spread should be biopsied and a surgical (local) stage assigned to both kidneys. Primary excision of the tumor masses should not be attempted, but rather patients should be given preoperative chemotherapy appropriate to the stage and histology of the tumors. Initial treatment is with the combination of vincristine and dactinomycin if the renal tumors are favorable histology and not more extensive than stage II (see Fig. 30-9 and Fig. 30-10). Those with more extensive, favorable histology disease would receive the combination of dactinomycin, vincristine, and doxorubicin, and those with anaplastic histology would receive vincristine, doxorubicin, cyclophosphamide, and etoposide (see Fig. 30-9 and Fig. 30-10).

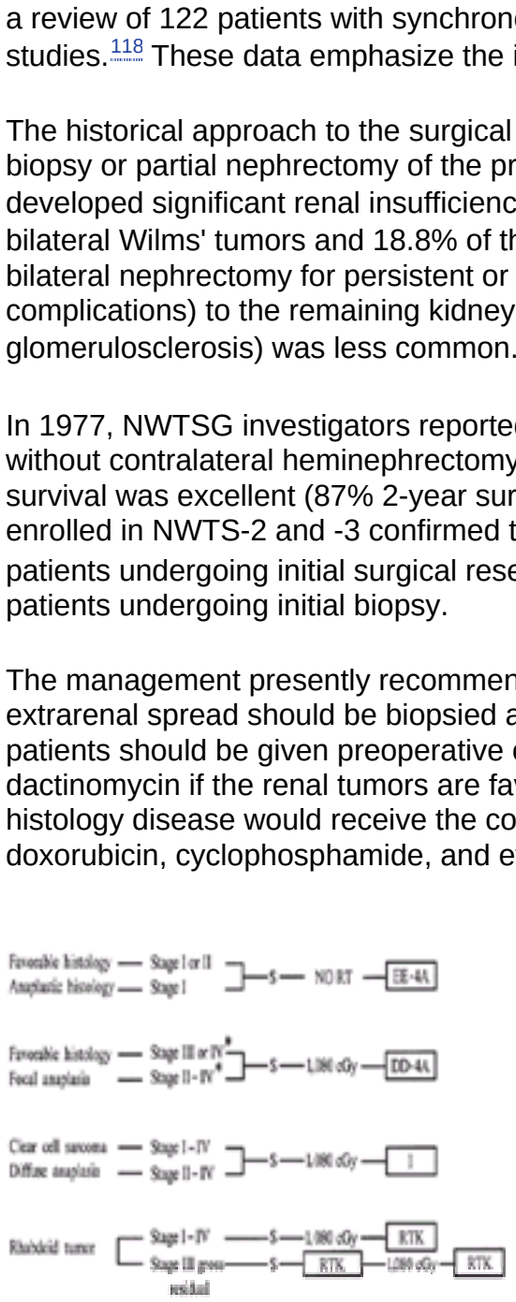

**FIGURE 30-9.** Treatment algorithms by tumor histology and stage used on National Wilms' Tumor Study 5. Stage IV patients (*) receive abdominal radiation therapy (RT) depending on the local stage of the abdominal primary. S, surgery.

A reevaluation is performed at approximately week 5 to determine if there has been sufficient response of the tumors to allow tumor resection, with preservation of a substantial amount of normal renal tissue. Experience in SIOP has shown that maximal shrinkage occurs after 4 to 6 weeks of chemotherapy. Although a longer course of preoperative chemotherapy may not benefit a patient with a unilateral tumor, that is not always the case with a bilateral Wilms' tumor wherein even a modest further reduction in tumor size may be helpful. This is particularly true for centrally located tumors for which a 1-cm reduction in size may allow partial nephrectomy with sparing of the renal hilum. Additional chemotherapeutic agents, such as doxorubicin, with or without radiation therapy, may be necessary for the management of children whose tumors respond poorly to the combination of vincristine and dactinomycin.

In any event, a second look procedure is recommended when serial imaging studies show no further reduction in the tumor. Failure of the mass to shrink is not always

due to persistent viable tumor. There may be necrosis, fibrosis, or persistence of skeletal muscle or differentiated elements within the original lesion. [218] A biopsy of the kidney may be necessary to confirm persistent viable tumor. At the time of the second look procedure, partial nephrectomy or wedge excision of the tumor should be considered, but only if complete tumor resection with negative margins can be obtained and part of either or both of the kidneys can be salvaged. Radical nephrectomy is performed when the extent of the tumor precludes salvage.

### Partial Nephrectomy

The occurrence of renal failure, presumably due to hyperfiltration injury, in occasional patients following treatment for unilateral Wilms' tumor [219,220] has led some investigators to evaluate the potential for renal parenchymal-sparing surgery. The data regarding renal damage from hyperfiltration following unilateral nephrectomy is conflicting. After treatment for unilateral Wilms' tumor, some patients may develop proteinuria and a reduced creatinine clearance. [221,222]However, other investigators have not confirmed these findings.[223,224]A recent review of NWTSG patients with unilateral Wilms' tumor identified the incidence of renal failure as 0.25% with a median follow-up of 6 years.[216] Most of these patients had the Denys-Drash syndrome with intrinsic renal disease, and generally presented with renal failure or progressed to end-stage renal disease. Another risk factor for the development of renal failure was irradiation of the remaining kidney, which is now given to few children and at a markedly reduced dose. Therefore, the risk of developing renal failure after current treatment for unilateral Wilms' tumor appears to be quite low, although continued long-term follow-up of this cohort of children is necessary.

The majority of Wilms' tumors are too large to consider partial nephrectomy at initial presentation. Using stringent diagnostic imaging criteria—including (a) tumor limited to one pole and occupying less than one-third of the kidney; (b) preserved renal function in the involved kidney; (c) no tumor invasion of the collecting system or the renal vein; and (d) clear margins between the tumor, kidney, and surrounding structures—investigators from St. Jude Children's Research Hospital determined that only 4.6% (2 of 43) of patients would be eligible for partial nephrectomy at the time of diagnosis. [225]

Therefore, pretreatment with chemotherapy is usually required if renal-sparing surgery is to be considered, [226,227 and 228] remembering that postchemotherapy staging is inaccurate. After preoperative chemotherapy, as many as 10% to 15% of patients may be amenable to partial nephrectomy. [226,227 and 228] Some advocate enucleation of the tumor to allow parenchymal-sparing procedures for even centrally located tumors in which partial nephrectomy with a rim of renal tissue would be inadvisable. [227] The efficacy of this approach remains unclear, however.[229]

The possible benefits of renal parenchymal-conserving surgery must be evaluated against the potential risks of such procedures. There are surgical complications unique to partial nephrectomy, including urine leak and ischemic injury if cross clamping of the vessels is required. The failure to include nephrogenic rests in the surgical resection, identified in 28% of unilateral Wilms' tumor specimens, might increase the risk of intra-abdominal tumor recurrence or metachronous tumor in patients who have an excellent prognosis with current treatment approaches.

In summary, parenchymal-sparing renal surgery for patients with unilateral Wilms' tumor is controversial. The current recommendation of the NWTSG is to consider partial nephrectomy only for patients with a solitary kidney, renal insufficiency, bilateral Wilms' tumor, and possibly for those with Wilms' tumor–predisposing syndromes.

## GENERAL RADIOTHERAPEUTIC CONSIDERATIONS

Pioneering radiation oncologists noted that Wilms' tumors were responsive to radiation therapy, and this modality then became routine postoperative treatment at the Boston Children's Hospital, at which many of the initial observations concerning the management of these children were made. [230] Two methods were used at that institution to define the treatment volume. First, the portal was extended across the midline to include the entire circumference of the implicated vertebral bodies. [231] This was done to equalize the growth suppression; irradiation of only one side of a vertebra had been shown to lead to an obligatory scoliosis convex away from the irradiated side. Concern for late treatment complications obviously was much in the minds of the early workers, notably M. H. Wittenborg. Second, a preoperative excretory urogram was used to define the location and size of the kidney and its associated mass, considered to be the tissues of the original tumor bed. The upper, lateral, and lower limits of the field were thus defined. The dosage was age-adjusted, with infants receiving lower doses than 3- or 4-year-olds, because radiation effects on their normal tissues would result in more serious damage.

These radiation therapy concepts have been modified as the result of the clinical trials conducted by the NWTSG. For example, the age-adjusted dosages were shown to be unnecessary in tumors of favorable histology. The advent of effective drugs had a profound impact not only on the general management of these children but also on the indications for the administration of postnephrectomy abdominal irradiation. The first NWTSG randomized clinical trial indicated that presumed microscopic residual disease in the tumor bed of children with stage I/favorable histology Wilms' tumor can be successfully treated with combination chemotherapy rather than flank irradiation. Retrospective analyses of the data accumulated in NWTS-1 and NWTS-2 were conducted to determine the patterns of relapse and to evaluate the relationship between abdominal radiation therapy dose and intra-abdominal tumor recurrence. [232,233 and 234] In NWTS-3 the nonirradiated and irradiated (2,000 cGy) stage II/favorable histology patients had similar relapse-free survival percentages, as did those with stage III/favorable histology who received nominal doses of 1,000 versus 2,000 cGy. Meanwhile, excellent results continued to be recorded for stage I/favorable histology patients, none of whom received radiation therapy.[199]

In summary, NWTS-1, -2, and -3 demonstrated that stage I and II patients with favorable histology tumors who receive vincristine and dactinomycin do not require postoperative irradiation, and that a dose of 1,000 cGy is sufficient for local control in stage III/favorable histology patients if they also received chemotherapy with vincristine, dactinomycin, and doxorubicin. [235]

Whole lung irradiation (1,200 cGy) has been recommended for patients who present with pulmonary metastases visible on plain chest radiographs. A pilot study conducted by investigators from the SIOP produced similar outcomes to those of the NWTSG [179] in stage IV/favorable histology patients following treatment with nephrectomy and chemotherapy only.[236] Only patients with persistent or recurrent lung nodules received whole lung radiation therapy or surgical removal of the metastatic lesion(s). However, the United Kingdom Children's Cancer Study Group, using a similar approach, reported results that were inferior to those of the NWTSG in this group of patients.[237] The adverse effects of whole lung irradiation and chemotherapy as used in the NWTSG treatment regimens included radiation pneumonitis or *Pneumocystis carinii* pneumonitis. These complications are important causes of morbidity and mortality in patients with stage IV Wilms' tumor, [238] although *P. carinii* pneumonitis can be prevented with administration of prophylactic trimethoprim/sulfamethoxazole. It is recommended that chemotherapy doses given immediately after the completion of whole lung irradiation be decreased by 50% to reduce the incidence of radiation pneumonitis. Additional studies are needed to evaluate the need for whole lung radiation therapy in this group of patients.

Patients with pulmonary lesions identified on CT of the chest, but not on the chest radiograph, should undergo biopsy of one or more lesions to confirm that they are due to metastatic Wilms' tumor if treatment with whole lung irradiation and doxorubicin is planned. A report from St. Jude Children's Research Hospital suggested that patients with pulmonary metastases detectable only by CT have an increased risk of pulmonary recurrence after treatment with chemotherapy only, [173] but a retrospective review of the experience with similar patients treated on NWTS-3 did not demonstrate a clear benefit of whole lung irradiation. The 4-year relapse-free survival rate was 88.1% among 18 irradiated patients and 88.9% among nine nonirradiated patients. [175] Only a meticulously conducted randomized trial in which histologic confirmation of metastatic disease was mandatory could settle this issue.

## GENERAL CHEMOTHERAPY PRINCIPLES

Wilms' tumor was the first pediatric malignant solid tumor found to be responsive to the systemic chemotherapeutic agent dactinomycin. The use of dactinomycin for the adjuvant treatment of children with Wilms' tumor was pioneered by Farber. [239] Other active agents were identified subsequently, including vincristine, with a complete and partial response rate of 63% (17 of 27 patients); doxorubicin (Adriamycin), with a response rate of 60% (31 of 52 patients); and cyclophosphamide, with a complete and partial response rate of 27% (10 of 37 patients). [100]

The initial apparent success with dactinomycin led to other single-institution studies and cooperative group randomized trials to evaluate the use of adjuvant single-agent chemotherapy (Table 30-5).[100]

| | Chemotherapy | Radiation therapy |
|---|---|---|
| NWTS-1 | Vincristine and dactinomycin combination better for group I, II | Unnecessary for group I <2 years (if received chemotherapy) |
| NWTS-2 | Only 6 months vincristine and dactinomycin necessary for group I | Unnecessary for all group I |
| | Additional doxorubicin beneficial for groups II–IV | – |
| NWTS-3 | Only 11 weeks therapy necessary for stage I | – |
| | Doxorubicin unnecessary for stage II | Abdominal irradiation unnecessary for stage I |
| | Doxorubicin necessary for stage III | If use 1,000 cGy abdominal irradiation |
| | Doxorubicin unnecessary for stage III | If use 2,000 cGy abdominal irradiation |
| | Cyclophosphamide (as used) did not benefit stage IV | |
| NWTS-4 | "Pulse-intensive" chemotherapy as effective, less toxic and expensive | |
| | Only 6 months chemotherapy necessary for stages II–III | |

**TABLE 30-5. SUMMARY OF CONCLUSIONS FROM NATIONAL WILMS' TUMOR STUDY (NWTS) STUDIES 1 THROUGH 4 FOR PATIENTS WITH FAVORABLE HISTOLOGY TUMORS**

### National Wilms' Tumor Studies 1 and 2

In NWTS-1 (1969 to 1973), postoperative abdominal radiation therapy was shown to be unnecessary for the therapy of children younger than 2 years with group I Wilms' tumor who were treated with dactinomycin. The combination of vincristine and dactinomycin was shown to be more effective for the treatment of those with group II or III Wilms' tumor than either drug alone. [163]

The results of NWTS-2 (1974 to 1978) demonstrated that for children with group I disease, none of whom received abdominal irradiation, reducing the duration of chemotherapy with the combination of vincristine and dactinomycin to 6 months did not effect their prognosis adversely. The addition of doxorubicin to the combination of vincristine and dactinomycin, however, improved the relapse-free survival percentage of irradiated children with stages II to IV Wilms' tumor. [240]

### National Wilms' Tumor Study 3

The results of NWTS-3 confirmed that patients with stage I favorable histology Wilms' tumor can be treated successfully using an 11-week regimen composed of vincristine and dactinomycin without abdominal irradiation. The 4-year relapse-free survival percentage with this regimen was 89%, and the 4-year survival percentage was 95.6%.[199]

Patients with stage II favorable histology Wilms' tumor were randomized to receive vincristine and dactinomycin or these two drugs and doxorubicin. They were also randomized to receive tumor bed irradiation (2,000 cGy) or no radiation therapy. There was no statistically significant difference between the outcome for patients who were treated with vincristine and dactinomycin without abdominal irradiation compared with the results for patients on the remaining three treatment regimens. [199] Thus, both doxorubicin and abdominal irradiation were shown to be unnecessary for children with stage II favorable histology tumors.

Patients with stage III favorable histology Wilms' tumor were similarly randomized to treatment with vincristine and dactinomycin or these two drugs and doxorubicin. They were also randomized to receive 1,000 cGy or 2,000 cGy of abdominal irradiation. This study demonstrated that these patients benefitted from the addition of doxorubicin to the two-drug combination of vincristine and dactinomycin. Overall there was no statistically significant difference in the frequency of intra-abdominal relapse among those treated with 1,000 cGy compared to 2,000 cGy. However, there appeared to be a higher frequency of relapse among those treated with vincristine and dactinomycin with 1,000 cGy of irradiation (7 of 61) compared with those receiving vincristine and dactinomycin with 2,000 cGy of irradiation (3 of 68) or those receiving vincristine, dactinomycin, and doxorubicin with 1,000 cGy of irradiation (3 of 70). [235] This suggested the necessity of either doxorubicin or 2,000 cGy (rather than 1,000 cGy) of irradiation for treatment of stage III favorable histology patients.

Patients with stage IV Wilms' tumor were randomized to receive vincristine, dactinomycin, and doxorubicin, or these three drugs and cyclophosphamide. All underwent immediate nephrectomy, and all received abdominal irradiation and whole lung irradiation (1,200 cGy). There was no statistically significant improvement in relapse-free or overall survival percentage with the addition of cyclophosphamide to the three-drug regimen. [199]

### National Wilms' Tumor Study 4

Previous success in treatment strategies allowed the design of a unique study, NWTS-4, with the primary aims of continuing to improve treatment results while decreasing the cost of therapy through modification of the schedule of drug administration. This study was based on experimental [241] and clinical [242,243,244] and [245] data demonstrating the safety and efficacy of dactinomycin when administered in a single, moderately high dose.

The design of NWTS-4 (Fig. 30-8) allowed the results of “pulse-intensive” chemotherapy regimens (EE-4A, K-4A, DD-4A) using single doses of dactinomycin and doxorubicin to be compared with treatment regimens (EE, K, and DD) using traditionally divided-dose regimens of each drug. In addition, a reduction in treatment duration from approximately 15 months to 6 months was studied in patients with stages II to IV/favorable histology tumors.

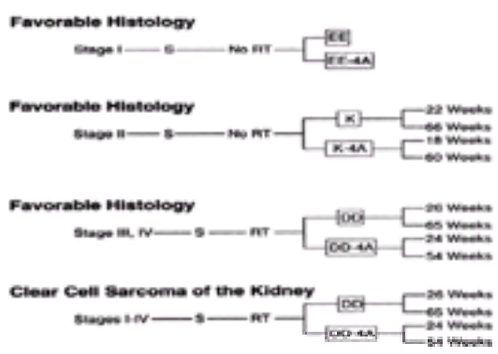


**FIGURE 30-8.** Treatment randomization for patients entered on National Wilms' Tumor Study 4. Doxorubicin and radiation therapy (RT) are included in the treatment regimen only for patients with stages III and IV Wilms' tumor. S, surgery.

Toxicity analyses confirmed that the pulse-intensive regimens actually produced less hematologic toxicity than the standard regimens, and that the administered drug dose intensity is greater on the pulse-intensive regimens. [246] In addition, an analysis of the cost of chemotherapy treatment suggested that at least $790,000 per year is saved if all U.S. children with stages I to IV/favorable histology Wilms' tumor are treated using the pulse-intensive regimens. [247]

Analysis of the results of the comparison between the standard (divided-dose) and pulse-intensive (single-dose) modes of chemotherapy administration demonstrated that there was no significant difference between the 2-year relapse-free percentages for patients treated with the pulse-intensive regimens (89.4%) and those treated with the standard regimens (90.5%).[248] Similarly, comparisons between the short and long treatment regimens suggest that they are equivalent for patients with stages II to IV favorable histology disease. [247] The results of the first four NWTS studies are summarized in Table 30-5.

## COMBINED-MODALITY THERAPY

These recommendations are based on the NWTSG experience and advocate early surgery without preoperative therapy for patients with apparently resectable unilateral disease with subsequent therapy according to stage and histology.

**Chemotherapy**

Many chemotherapy regimens appear to produce good results.[206,237,249] Those used by the NWTSG are detailed here because they have been tested in the largest numbers of patients. The treatments are modulated in intensity according to risk, based on stage and histology. An algorithm of treatments by stage and histology is shown in Figure 30-9 and the protocol details in Figure 30-10.

***Favorable Histology Wilms' Tumor***

For stage I and II disease, regimen EE-4A (Fig. 30-10) involves dactinomycin beginning within 5 days postnephrectomy (during week 0), and then at three weekly intervals, and vincristine beginning day 7 postnephrectomy once peristalsis has been established, then weekly for a total of 10 doses, then an increased dose with dactinomycin at weeks 12, 15, and 18. No radiation therapy is given.

For stage III and IV disease, regimen DD-4A (Fig. 30-10) includes four doses of doxorubicin in addition to dactinomycin and vincristine for 24 weeks. Postoperative irradiation is added for stage III primary tumors and for metastatic disease according to the relevant guidelines ( Table 30-6).

| Disease extent | Radiation volume | Dose[a] |
|---|---|---|
| Hilar lymph nodes<br>Gross or microscopic residual confined to flank | Tumor bed, crossing midline to include entire vertebral bodies | 1,080 cGy in six fractions |
| Para-aortic lymph nodes | Include entire length of bilateral paraaortic chains | 1,080 cGy in six fractions |
| Peritoneal seeding<br>Gross residual abdominal disease<br>Preoperative intraperitoneal rupture<br>Diffuse operative spill | Whole abdomen | 1,080 cGy in six fractions or 1,050 cGy in seven fractions |

[a]Local supplements of 1,080 cGy should be given to volumes greater than 3 cm in maximum diameter. A cone down field should encompass residual disease with a 1-cm margin. Greater than one-third of the contralateral kidney or the residual normal kidney in patients with bilateral Wilms' tumor should not receive doses higher than 1,440 cGy. Doses to more than one-half of the liver should not exceed 1,980 cGy.

**TABLE 30-6. DOSE AND VOLUME OF ABDOMINAL RADIATION FOR LOCAL STAGE III TUMORS**

***Anaplastic Wilms' Tumor***

Stage I tumors with focal or diffuse anaplasia are treated using regimen EE-4A as in stage I favorable histology tumors ( Fig. 30-10). It is currently recommended that stage II–IV tumors with focal anaplasia be treated the same as stage III favorable histology with regimen DD-4A and abdominal irradiation ( Fig. 30-10 and Table 30-6).

Patients with tumors having diffuse anaplasia that are greater than stage I have fared relatively poorly with the traditional Wilms' tumor agents dactinomycin, vincristine, and even doxorubicin. A newer regimen is under study in the NWTS-5, the details of which are shown in Figure 30-10, although no outcome data is yet available for this regimen. Previous studies demonstrated a benefit from the addition of cyclophosphamide to the standard three-drug regimen.[250] Cyclophosphamide has previously been used in relatively low doses for Wilms' tumor. Regimen I uses cyclophosphamide in high dose in conjunction with etoposide and continues the use of doxorubicin but in combination with cyclophosphamide. Whether this regimen improves the outcome for stages III and IV diffusely anaplastic disease, which have been particularly poor, remains to be demonstrated.

***Clear Cell Sarcoma of the Kidney***

The use of doxorubicin appears to be particularly effective in the treatment of clear cell sarcoma of the kidney.[123] Because the outcome for patients with clear cell sarcoma has remained inferior to those with favorable histology Wilms' tumor, patients on NWTS-5 are currently being treated with regimen I, as for anaplastic tumors, which uses increased doses of doxorubicin with the vincristine backbone but also introduces higher-dose cyclophosphamide and etoposide. As for children with anaplastic tumors, outcome results for children with clear cell sarcoma of the kidney treated with regimen I are not yet available.

***Rhabdoid Tumor of the Kidney***

No satisfactory treatment for this disease has been reported. A recent analysis of patients with rhabdoid tumor, however, suggests that although the outcome has been particularly poor for children younger than 1 year; older children with the disease may be more readily curable with traditional approaches (G. Tomlinson, *personal communication*, 2000). Patients with rhabdoid tumor of the kidney of all stages are being treated on the NWTS-5 with a new regimen, RTK ( Fig. 30-10), which includes carboplatin and etoposide as well as high-dose cyclophosphamide in a single-arm, phase II study. This study has not yet completed accrual.

**Radiation Therapy**

Megavoltage teletherapy apparatus or the equivalent is used, and daily doses of 180 cGy are delivered. The daily dose is reduced to 150 cGy when large volumes, such as the whole abdomen or the whole thorax, are included in the fields. Doses are specified as midplane values without correction for air transmission or bone absorption. The use of simulation and portal films is essential to ensure accurate beam direction.

Radiation therapy, when indicated, is initiated when the patient is stable postoperatively, free of ileus or diarrhea, and has a hemoglobin level of at least 10 g per dL. Retrospective analysis of the results from each of the first three completed NWTSG trials showed the prognostic importance of starting radiation therapy within 10 days of surgery.[233,234 and 235] Thus, arrangements should be made for treatment simulation while the pathology examination is pending so that irradiation can be started promptly if indicated, because it is usually easier to cancel such arrangements than to initiate them at the last minute.

***Favorable Histology Wilms' Tumor***

**Stages I and II**

No postoperative radiation therapy is recommended in children receiving dactinomycin plus vincristine according to regimens similar to those reported by the NWTSG.

**Stage III**

Doses and volume of radiation for abdominal tumors that are classified as stage III are shown in Table 30-6. The tumor bed is defined as the kidney and its associated lesion as they are visualized on preoperative imaging studies. The portal is always extended medially to include the entire vertebral column at the implicated levels (i.e., across the midline medially). The field is extended as needed to include the para-aortic chains when para-aortic nodes are found to be involved. The portal for whole abdominal irradiation includes all the peritoneal surfaces and extends from the domes of the diaphragm to the inferior margins of the obturator foramina. External beam blocks are introduced to shield the femoral heads.

### Stage IV

Postoperative abdominal irradiation is given only to patients whose primary tumor is classified as Stage III ( Table 30-6).

Recommendations for irradiation of metastatic sites are shown in Table 30-7 and discussed below. For bilateral lung irradiation, the entire thoracic cavity is irradiated without shields, except those protecting the humeral heads. The field extends from the apex of the lung to the posterior inferior recesses of the costophrenic sulci. The latter come to the bottom of T12 or lower in most children. Shielding of mediastinal structures is not recommended because marginal recurrences in mediastinal lymph nodes have developed in patients managed in this way. Infants are managed differently, as discussed in the section on special considerations.

| Disease site | RT field | RT dose |
|---|---|---|
| Liver | Involved portion plus 2-cm margin | 1,980 cGy* in 11 fractions |
| Lung | | |
| Age ≥18 mo | Bilateral lung | 1,200 cGy in eight fractions |
| Age <18 mo | Bilateral lung if no response to chemotherapy | 900 cGy in six fractions with 150-cGy supplements to specific nodules |
| Lymph nodes | Involved lymph nodes | 1,980 cGy in 11 fractions |
| Brain | Whole brain | 3,060 cGy in 17 fractions |
| Bone | Lesion plus 3-cm margin | 3,060 cGy in 17 fractions |

RT, radiation therapy.
*Local supplements of 540 to 1,080 cGy can be given to small portions of the liver containing residual gross tumor at the completion of the recommended 1,980 cGy.

**TABLE 30-7. TREATMENT OF METASTATIC DISEASE**

For liver metastases, only those that are unresectable at diagnosis are irradiated. The treatment portal includes that portion of the liver known to be involved, as visualized on CT or magnetic resonance imaging studies. The whole liver is treated in children with diffuse metastases. It is recognized that liver tolerance is approached by the doses recommended, especially in view of the radiation-enhancing and radiation-reactivating drugs (dactinomycin and doxorubicin) used in the management of these children. Extreme caution is therefore recommended, with careful monitoring of liver function tests and blood counts during treatment. A selective thrombocytopenia can appear in these patients, who nonetheless have a surprisingly good outlook after aggressive therapy. Breslow and coworkers [179] reported 72% survival at 4 years in NWTS-1 and -2 children with favorable histology tumors and liver lesions at diagnosis, with or without lung metastases. These findings were confirmed in an analysis of liver involvement at diagnosis in NWTS-3 patients. [251]

Metastases to bulky lymph nodes, brain, bone, or other areas are treated similarly to the liver, although with higher doses for the brain and bone ( Table 30-7). The whole brain is treated in daily doses of 180 cGy, but the entire bone need not be irradiated for skeletal metastases.

#### *Anaplastic Wilms' Tumor*

Patients with tumors classified as stage I with focal anaplasia are treated exactly as those with favorable histology. NWTSG analyses have also shown that children with stage I diffusely anaplastic tumors have the same outlook as those with stage I/favorable histology lesions when treated similarly. [252] Therefore these children should also receive combination chemotherapy only, with vincristine and dactinomycin, and no postoperative radiation therapy. Tumors with focal or diffuse anaplasia that are stage II or III, however, require more aggressive therapy and postoperative irradiation as is given for stage III favorable histology ( Table 30-6).[250]

Metastatic sites in patients with tumors with diffuse anaplasia should be treated the same as for stage IV/favorable histology disease ( Table 30-7), except those in the liver, for which doses of 3,060 cGy in 18 fractions are given, with 540 to 1,080 cGy supplements permissible to small residual volumes.

#### *Clear Cell Sarcoma of the Kidney*

All patients, regardless of stage, are recommended to receive postoperative irradiation. The abdomen should be treated as outlined in Table 30-6, even with stage I disease, and metastatic sites should be treated as outlined in Table 30-7.

#### *Rhabdoid Tumor*

All patients with rhabdoid tumor of the kidney are also currently recommended to receive irradiation according to Table 30-5 and Table 30-6, although in the current NWTS-5 studies this irradiation is delayed until week 6 to allow determination of initial response to chemotherapy.

## SPECIAL CONSIDERATIONS

### Neonates and Infants

Wilms' tumor is rarely found in neonates.[253,254] Newborns and infants younger than 11 months require reduction of chemotherapy doses to 50% of those used in older children, even after conversion of amounts calculated on the basis of surface area to the dose per kilogram. Infants with pulmonary metastases pose particular problems. The NWTSG committee recommends that thoracic irradiation be given only to those children younger than 18 months who do not have complete resolution of metastases within 4 weeks of initiating chemotherapy. Children with residual nodules should receive 900 cGy in 150-cGy daily doses with a single 150-cGy supplement to nodules that do not disappear after 900 cGy ( Table 30-7). Irradiation is recommended, because evidence from relapsed NWTS-3 patients suggests that the best relapse-free survival is obtained in children who had thoracic irradiation added to chemotherapy. [255] An alternative to irradiation is surgical excision of lesion(s) that remain after the first cycle of dactinomycin, vincristine, and doxorubicin (all such children should be on three-agent chemotherapy). Another alternative is to reserve surgical excision for children who have residual masses after the delivery of 900 cGy to the whole chest. The efficacy of these operative approaches has not been tested systematically, however. The NWTSG reported no difference in survival for relapsed patients with a solitary metastasis who did or did not have the lesion removed surgically.[256]

### Extrarenal Wilms' Tumor

Though rare, extrarenal Wilms' tumors can be found in the pelvis and even the thorax as well as the retroperitoneal space. [103,257] The same general therapy precepts can be followed as when the kidney is affected, although it may be prudent to use the guidelines for chemotherapy and radiotherapy for stage III tumors under these unusual circumstances.

### Horseshoe Kidneys

Thirteen cases of Wilms' tumor arising in a horseshoe kidney were found among 2,961 (0.4%) patients reviewed by the NWTSG. [258] Seven of the thirteen had stage I or II disease. Treatment was according to the guidelines for comparable-stage unilateral disease, with an overall survival of 85% (mean follow-up, 32 years). These findings indicate that the tumors are no more aggressive than unilateral tumors. By extension, patients with tumors in discoid kidneys can be managed successfully using preservative approaches.

### Recurrent Disease

Children with relapsed, favorable histology Wilms' tumor have a variable prognosis, depending on their initial stage, the site of relapse, the time from initial diagnosis

to relapse, and their previous therapy. Favorable prognostic factors include no prior treatment with doxorubicin, relapse more than 12 months after diagnosis, and subdiaphragmatic relapse in patients not previously given abdominal irradiation. [255] Children in this more favorable group should be treated aggressively, because they generally have a good response to retrieval therapy. Although surgical excision of pulmonary metastases does not improve outcome, [256] surgical biopsy or excision of recurrence should nonetheless be performed to histologically confirm the presence of recurrent disease, particularly in the case of intra-abdominal recurrence, to reduce the tumor burden before the initiation of radiation therapy and combination chemotherapy.

The optimal chemotherapy regimen has not been defined but should include doxorubicin, if not used previously. The combination of etoposide and carboplatin [259] is active against recurrent Wilms' tumor with favorable histology and, although studied in few cases, probably with diffuse anaplasia and in clear cell sarcoma of the kidney. There are insufficient studies of this combination in patients with rhabdoid tumor.

The combination of etoposide and ifosfamide is also highly active in favorable histology Wilms' tumor and clear cell sarcoma, [260,261] although the nephrotoxicity of ifosfamide in children with Wilms' tumor discourages its use in previously untreated children. [261,262] The substitution of cyclophosphamide, using a dose intensity similar to that used in the phase II trial of ifosfamide and etoposide, may also be effective [263] and is currently under study in NWTS-5.

Patients who relapse after prior treatment with a regimen that included doxorubicin or who develop a recurrence in the abdomen (including liver) after previous irradiation have a poor prognosis. It has been suggested that high-dose chemotherapy with stem cell rescue should be used in the management of patients with adverse prognostic factors at the time of relapse,[264,265] although the relative efficacy of this approach remains unproved. In general, these children should be referred to centers that are conducting research in the treatment of children with recurrent solid tumors.

## FOLLOW-UP DURING AND AFTER THERAPY

Children treated for Wilms' tumor should be examined regularly by a physician who is familiar with the natural history of this tumor and the complications of therapy. Careful palpation of the abdomen will help detect local tumor recurrence, tumor growth in the liver, or contralateral tumor development. Suspicious findings on physical examination should be confirmed or clarified using abdominal ultrasound or CT. Lung irradiation may affect the thyroid gland, which should therefore be palpated yearly for life because of the known association between irradiation and thyroid neoplasms. In addition, these patients should have thyroid function tests performed at yearly intervals for 5 years to detect possible hypothyroidism.

A routine schedule for diagnostic imaging follow-up is shown in Table 30-8. Although no studies have been carried out to evaluate the most effective schedule of follow-up, these proposed intervals are based on the fact that most recurrences (approximately 90%) occur in the first 2 years after diagnosis, and virtually the remainder occur in the next 2 years.[199,247] Subsequent imaging studies should be obtained as clinically indicated.

| Imaging study | Patient | Schedule |
|---|---|---|
| Chest x-ray or computed tomography scan of thorax | All patients | Every 6 wk until complete remission is documented; every 3 mo × 8, followed by every 6 mo × 4 |
| Abdominal ultrasound | Patients with nephrogenic rests | Postoperatively after 6 wk and 3 mo then every 3 mo until age 8 yr |
| | Patients without nephrogenic rests | Postoperatively after 6 wk and 3 mo; then every 3 mo × 7, followed by every 6 mo × 4 |

**TABLE 30-8. IMAGING STUDIES RECOMMENDED FOR FOLLOW-UP OF CHILDREN WITH FAVORABLE OR ANAPLASTIC HISTOLOGY WILMS' TUMOR**

The first site of disease recurrence with all stages is most frequently the lung. Lung metastases are best seen on CT, although not every lesion detected by CT represents metastatic disease. Abdominal ultrasonography is obtained for the early detection of infradiaphragmatic relapses, which is a greater issue for stage II and III tumors. Patients who at diagnosis present with hematogenous metastasis (brain, lung, liver, and bone) require evaluation of the affected sites at similar intervals to those recommended for the chest and abdomen.[266] The brain is most easily evaluated using magnetic resonance imaging. Although plain radiographs may be suitable to follow the progress of a specific bone lesion, a radionuclide bone scan should be obtained in patients whose presentation suggests bony involvement and in all patients with clear cell sarcoma. Consideration should be given to longer follow-up for clear cell sarcoma patients because relapses are known to occur for as long as 5 years postdiagnosis.

In children with any of the Wilms' tumor–predisposing syndromes or with nephrogenic rests in one or both kidneys, ultrasonography of the remaining kidney is performed for a longer period because the opposite kidney continues to be at risk for several years ( Table 30-8). This is particularly true for children younger than 12 months at diagnosis.[118]

Patients with mesoblastic nephroma require fewer studies, however, because distant metastases are rare. [267,268 and 269] Infradiaphragmatic recurrence may occur, however, particularly in the setting of intraoperative tumor rupture, and ultrasonographic studies should be obtained every 3 months for 18 months postnephrectomy.

Irradiated bony structures are monitored for life for possible radiation-associated neoplasms. Radiographs of irradiated bony structures (e.g., lumbar spine, pelvis, and ribs) are taken annually until full growth is attained. Thereafter they are obtained every 5 years indefinitely. This regimen is advised for the early detection of second neoplasms.[266] Patients receiving pulmonary or even abdominal radiotherapy for Wilms' tumor may have abnormalities of pulmonary function, and therefore pulmonary function tests should be considered as part of their long-term follow-up.

## LONG-TERM COMPLICATIONS AND FOLLOW-UP OF THERAPY

Because Wilms' tumor is usually a curable malignancy, it is essential to limit iatrogenic sequelae. Although the damage from the primary treatment, nephrectomy, may be limited to the kidney, additional treatment modalities may cause acute and chronic damage to several organs, such as the heart, lungs, liver, bones, and gonads. In addition, both chemotherapeutic agents and radiation therapy can induce second malignant neoplasms (SMNs).

### Renal Function

Nephrectomy, the use of nephrotoxic agents (primarily chemotherapy and certain antibiotics), and radiation therapy to the remaining kidney can potentially affect renal function in children treated for Wilms' tumor. However, the NWTSG experience suggests that serious renal dysfunction is not common in this patient population. [216] Of 5,823 children evaluated, of whom 451 had bilateral disease, a total of 55 patients (approximately 1%) were identified with renal failure. Thirty-nine of the fifty-five (71%) children had bilateral disease in which the most common cause of renal failure was surgery (bilateral nephrectomy) followed by radiation-induced damage and postsurgical complications in the remaining kidney. For patients with unilateral Wilms' tumor and an apparently normal contralateral kidney, the risk of renal failure was very low (0.25%) and the most common cause was unrecognized renal disease (Denys-Drash syndrome) followed by radiation nephritis. [216] It is noteworthy, however, that many of these children with unilateral Wilms' tumor are still young. They therefore remain at risk for renal deterioration as time goes on. Indeed, patients have been described with proteinuria and hypertension developing 10 to 20 years after nephrectomy, local irradiation, and chemotherapy for unilateral Wilms' tumor.[220]

### Cardiac Function

Up to 25% of Wilms' tumor patients treated with doxorubicin can be expected to develop some cardiac abnormality, most frequently signs of increased left ventricular

afterload.[270] Risk factors include both the cumulative dose and dose intensity of anthracycline. [270] The cumulative percentage of children treated with doxorubicin on NWTS-2 and -3 who actually developed congestive heart failure was 1.7% at 15 years postdiagnosis. [271] The onset of heart failure occurred 1.3 to 11.7 years after diagnosis, with 50% occurring after 8 years. [271] Whole lung irradiation is an additional risk factor. The percentage of children who developed congestive heart failure after treatment in NWTS-2 and -3 increased from 1.0% among those whose treatment did not include whole lung irradiation to 5.4% among those whose treatment did.[271] There have also been reports of congestive heart failure in patients who received left ventricular irradiation from the abdominal radiation therapy for a left sided tumor.[272]

### Pulmonary Function

Radiation therapy, as well as certain chemotherapeutic agents, can cause serious changes in pulmonary function. [273] Review of patients with metastatic pulmonary disease entered on NWTS-3, revealed “pneumonitis of unknown etiology” (likely radiation pneumonitis) in approximately 12% of patients after pulmonary irradiation.[238] The mortality associated with pneumonitis of unknown etiology was high; only 25% of patients survived. [238] By contrast, all children with pneumonitis due to *P. carinii* survived the episode. The incidence of pneumonitis has been reduced by the reduction in doxorubicin and actinomycin dosage when given concurrently with irradiation.

### Hepatic Function

The liver may be damaged by several cytotoxic agents, including dactinomycin and irradiation. [274] Although most early reports suggested that hepatic irradiation was the most important factor, recent publications have documented hepatic toxicity after the use of vincristine and dactinomycin in nonirradiated children with Wilms' tumor.[275,276] In NWTS-4, the incidence of significant hepatotoxicity ranged from 2.8% to 14.3% in groups that received different doses and schedules of dactinomycin, suggesting a dose-related toxicity.[275] Investigators in Europe have reported a similar incidence of hepatotoxicity (12.5%) on SIOP-9. [276]

Hepatic veno-occlusive disease (VOD) is primarily a clinical diagnosis with the following features: hepatomegaly, right upper-quadrant pain, jaundice, ascites, and unexplained weight gain. VOD, which occurs within the first 10 weeks of therapy, has been diagnosed in children with Wilms' tumor that undergo initial nephrectomy, as well as in those receiving chemotherapy before surgery. [276] In the latter group, hepatotoxicity occurred during preoperative chemotherapy as well as during postnephrectomy therapy. Although treatment for VOD is primarily supportive, chemotherapy does not need to be withheld once the signs of VOD have disappeared. Long-term or permanent hepatic dysfunction has not been a problem for Wilms' tumor survivors.

### Bone Marrow

The frequency of severe hematologic toxicity was evaluated in patients treated with the divided-dose or the single-dose regimens in NWTS-4. The single-dose regimens produced less hematologic toxicity, despite the fact that the myelosuppressive drugs were administered at a higher dose intensity. [246]

Morgan and colleagues[277] reported severe hematologic toxicity among 47% of infants younger than 12 months after treatment with full doses of chemotherapeutic agents, compared with 13% of similar patients treated with 50% of the protocol drug doses. The use of lower drug doses did not increase the frequency of relapse among patients younger than 12 months.[277] These data confirmed the results of other investigators[278,279] and led to the recommendation that all infants receive only 50% of the usual dose of any chemotherapeutic agent. [280]

### Gonadal Function

Infertility is one of the main late sequelae of cytotoxic chemotherapy. Because dividing cells are most sensitive to the cytotoxic effects of alkylating agents, the impairment of gonadal function is more frequent in boys than in girls. [281,282] Because most patients with Wilms' tumor are not treated with alkylating agents, gonadal dysfunction is primarily of concern for those that receive these drugs after relapse.

Women who received whole abdominal irradiation in childhood may develop ovarian failure, [283] but this may be prevented by moving both ovaries out of the irradiation field.[284] Several investigators have reported that pregnancy outcomes are adversely affected by abdominal irradiation for Wilms' tumor. [87,88,285] In the largest study thus far, women irradiated for Wilms' tumor had an increased perinatal mortality rate (relative risk of 7.9) and an excess of low-birth-weight infants (relative risk of 4.0) when compared with white women in the United States.[88] By contrast, an adverse outcome was found in only 2 (3%) of the 77 pregnancies in nonirradiated girls with Wilms' tumor or in wives of boys with Wilms' tumor. [88] The high-risk nature of pregnancy should be considered in the counseling and prenatal care of women who have received abdominal radiotherapy for Wilms' tumor.

### Musculoskeletal Function

The late effects of trunk irradiation—scoliosis and soft tissue underdevelopment—are still being seen despite the advent of megavoltage irradiation. [286,287,288 and 289] One would expect the least deformity, of course, when radiation is omitted entirely. That expectation has been documented by the NWTSG, which recorded 66 instances of “musculoskeletal” difficulties (not otherwise defined) among 88 irradiated stage I children followed for 5 or more years, versus 7 in 93 unirradiated cohorts. [290] A radiation dose greater than 20 Gy is associated with a greater risk of major morbidity in the spine or any irradiated bone. [291]

### Second Malignant Neoplasms

Wilms' tumor survivors are at risk for SMNs, possibly due both to inherited predisposition and to treatment such as chemotherapy and radiotherapy. The NWTSG experience reveals a cumulative incidence of SMN of 1.6% 15 years after the initial diagnosis of Wilms' tumor. [292] The most important risk factor for the occurrence of an SMN in this cohort was treatment with radiation therapy. Initial treatment that included doxorubicin further increased this risk. Significantly, though, even those whose therapy included only dactinomycin and vincristine, without radiation therapy, had an increased risk of cancer when adjusted for age, sex, and race, compared to the U.S. population.[292] Treatment for relapse further increased the risk for a SMN by a factor of four to five. [292] The experience in Europe has been similar.[293] Compared to the general population, survivors of Wilms' tumor had a fivefold increased risk of developing an SMN in the first 10 years from diagnosis. [293]

### Long-Term Follow-Up

These data demonstrate the importance of the continued efforts to limit the use of intensive therapy in the treatment of patients thought to be at low risk for recurrence. Intensive therapy is now applied only to patients with advanced-stage disease or those with tumors displaying unfavorable histologic features.

Ongoing care for Wilms' tumor survivors should include screening for complications that can be expected based on the therapeutic regimen used. Many of these possible long-term sequelae can be detected by a simple protocol that includes history, physical examination, assessment of renal function, urinalysis, and echocardiogram as indicated. The most important factor is knowledge of the possible adverse outcome. This will allow for the implementation of early intervention if needed.

## FUTURE CONSIDERATIONS

Major progress has been made in the management of children with Wilms' tumor. More than 85% can be cured by current therapies. Future treatment research must address several important issues. The most important of these is the need to identify new prognostic markers to distinguish those patients who require more effective therapy for cure from those who could potentially be cured without agents that are associated with adverse sequelae, such as radiation or doxorubicin. Such factors may be biological, genetic, or traditional histologic factors to better predict for local recurrence—distinguishing current stage II patients who relapse without irradiation from stage III patients that are irradiated unnecessarily, for example.

The role of whole lung irradiation in the management of patients with stage IV/favorable histology tumors requires further clarification. Although not all such patients require whole lung irradiation, it is not yet possible to clearly distinguish those that do. It may even be possible with emerging technical advances, such as

intensity-modulated radiotherapy, to restrict whole lung fields to small, highly conformal fields, minimizing the amount of lung tissue irradiated.

The efficacy of chemotherapeutic agents other than anthracyclines in combination with vincristine and dactinomycin for the treatment of patients with stage III or IV/favorable histology tumors with a high risk of relapse must be studied. Combinations currently being studied in relapsed patients such as cyclophosphamide/etoposide and carboplatinum/etoposide show promise in this regard.

Other problems that need to be carefully evaluated include the use of renal-sparing surgical procedures in the management of patients with bilateral Wilms' tumor and those with conditions known to predispose to the development of metachronous Wilms' tumor. It is still hoped that characteristics can be identified that define those with a negligible risk of recurrence for whom nephrectomy only might be an appropriate treatment approach.

Evolving understanding of the genesis of Wilms' tumor at the genetic level may allow an increased ability to identify individuals at highest risk for development of the disease: the predisposed members of a family segregating the tumor or the subset of children with a Wilms' tumor–predisposing condition who will actually develop a tumor.

Finally, the best treatment for Wilms' tumor will not likely always be chemotherapy as we know it. Emerging treatments such as the antiangiogenic factors will likely be actively explored in the near future.

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# 31

# NEUROBLASTOMA

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## INTRODUCTION

The neuroblastic tumors (including neuroblastoma, ganglioneuroblastoma, and ganglioneuroma) are derived from primordial neural crest cells, that ultimately populate the sympathetic ganglia, adrenal medulla, and other sites. The variations in tumor locations and degrees of histopathologic differentiation result in an array of diverse clinical and biologic characteristics and behavior. [1,2] and [3] In addition to demonstrating spontaneous regression as well as differentiation to benign neoplasms, these tumors also exhibit extremely malignant behavior when observed in older children with regional or disseminated disease.

In the last 30 years, there has been substantial improvement in the outcome of infants as well as older children with local or regional neuroblastoma. Indeed, improvement has also occurred in the outlook for older children with metastatic disease at diagnosis. The ever-increasing ability to distinguish prognostic subsets of patients based on clinical and biologic features has allowed a better understanding of relative risk for recurrent disease and, therefore, the development of risk-related therapy that is not based solely on age and stage. In the face of these advances, age still remains an important indicator of outcome. With current treatment, the risk distinction seems most clear between infants, defined hereafter as 1 year or younger, and older children.

This chapter reviews the current understanding of the prognostic implications of various clinical and biologic features of neuroblastoma and relates these characteristics to current trends as well as future strategies for treatment.

## EPIDEMIOLOGY

### Incidence

Neuroblastoma is the most common extracranial solid tumor in children, accounting for 8% to 10% of all childhood cancers. The prevalence is approximately 1 case per 7,000 live births, and there are approximately 600 new cases of neuroblastoma per year in the United States. [3,4] and [5] This corresponds to an incidence of 10.4 per million per year in white children and 8.3 per million per year in black children younger than 15 years. Evidence indicates that this incidence is fairly uniform throughout the world, at least for industrialized nations. The tumor is slightly more common in boys than in girls, with a male to female sex ratio of 1.1:1.0 in most large studies.

Neuroblastoma is almost exclusively a pediatric neoplasm and is the most common cancer diagnosed during infancy. [4] Review of 3,059 neuroblastoma patients registered on cooperative group studies at POG institutions from February 1990 to February 2000 (n = 2,148) and at CCG institutions from August 1991 to August 1995 (n = 911) showed a median age at diagnosis of 17.3 months. In this cohort, 40.1% of patients were diagnosed as infants, 89.4% at ages younger than 5 years, and 97.8% by 10 years of age (Fig. 31-1). Of note, past estimates of the median age at diagnosis for neuroblastoma patients were approximately 22 months and suggested a bimodal distribution. [5] The earlier median and exponential decrease of age at diagnosis noted here may be due to either (a) earlier diagnosis as a result of better awareness or improved diagnostic imaging, or (b) patients with high-risk disease, who are generally older at diagnosis, not being consistently registered on

POG and CCG cooperative group trials during the years reviewed.

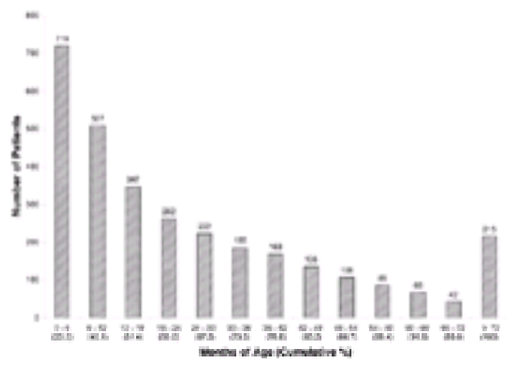


**FIGURE 31-1.** Age at diagnosis in 3,059 neuroblastoma patients. (Courtesy of W.B. London, Pediatric Oncology Group Statistical Office, Gainesville, FL.)

### Environmental Studies

The etiology of neuroblastoma is unknown in most cases, but it seems unlikely that environmental exposures play a major role. There have been a few reports that have implicated the following intrauterine exposures: alcohol (fetal alcohol syndrome), seizure medications, diuretics, neurally active drugs (such as tranquilizers and prescription pain medications), fertility drugs or hormones, and maternal use of hair coloring products. [6,7,8,9,10,11 and 12] However, none of these have been seen consistently or confirmed by larger studies. There have also been reports of an association with a variety of parental occupations, including certain electrical, farming, gardening, and painting jobs, suggesting parental environmental exposures may have contributed to neuroblastoma in their offspring, but none has been seen consistently.[13,14,15 and 16] Finally, there has been a recent report that BK virus infection may play a role in the pathogenesis of neuroblastoma, [17] but this has not yet been confirmed. At present, therefore, although limited studies have implicated a variety of prenatal exposures and parental occupations in neuroblastoma causation, in general the associations are weak. Thus, no prenatal or postnatal exposure to drugs, chemicals, viruses, or radiation has been associated strongly, consistently, or unequivocally with an increased incidence of neuroblastoma. This does not preclude a possible role of environment in the pathogenesis of neuroblastomas, but to date no strong environmental exposure or factor has been identified.

## GENETICS

### Constitutional Chromosomal Abnormalities and Associated Conditions

Germline chromosomal abnormalities have been seen rarely in children with neuroblastoma, but such abnormalities can facilitate the identification of a predisposing gene. Chromosome band 1p36 is a frequent site of somatic deletion in neuroblastoma cells, and three neuroblastoma patients have been described with germline interstitial deletions of 1p36. [18,19 and 20] Each patient was diagnosed with neuroblastoma during infancy and had profound neurocognitive deficits. The constitutional deletions overlap the location of a putative 1p36 tumor suppressor gene (see below), suggesting that germline absence of a gene within this region may predispose to the development of neuroblastoma. Constitutional balanced translocations involving 1p have also been identified in two additional infants with neuroblastoma. [21,22] These translocations involved the long arm of chromosome 17 [t(1;17)(p36;q12-21)] in a patient with localized disease and the long arm of chromosome 10 [t(1;10)(p22;q21)] in a patient with stage 4S disease. A gene located at chromosome band 1p22 ( *NB4S*) was disrupted by the latter translocation, suggesting that inactivation of this gene may play a causal role in neuroblastoma tumorigenesis. [23] At least 14 other cases of constitutional chromosomal rearrangements in neuroblastoma patients have been identified, [24] but the lack of a consistent pattern indicates that many of these rearrangements may be coincidental rather than causal.

Neuroblastoma occurring coincident with congenital anomalies is uncommon. One study showed a higher incidence of neurodevelopmental abnormalities in the brains of children dying of neuroblastoma, but the significance of these findings is unclear. [25] One recent review suggested a higher incidence of neuroblastoma in girls with Turner's syndrome,[26] although this has not yet been independently confirmed. Interestingly, another study suggested a lower incidence of neuroblastoma among patients with Down syndrome.[27] Hirschsprung's disease, central hypoventilation (Ondine's curse), and NF1 have all been described in both sporadic and familial neuroblastoma patients, suggesting the existence of a global disorder of neural crest–derived cells (neurocristopathy). [28,29,30,31,32,33,34,35 and 36] Indeed, two studies have documented *NF1* gene mutations in a subset of neuroblastoma cell lines,[37,38] and a patient has been reported with NF1 and neuroblastoma whose tumor had a homozygous deletion of the *NF1* gene.[39] Epidemiological evidence suggests that the association of neuroblastoma with NF1 is merely coincidental, [36] however, and familial neuroblastoma is not linked to the *NF1* locus, even in a family in which the proband had both conditions. [35] Thus, germline inactivation of *NF1* does not appear to predispose to neuroblastoma, but somatically acquired inactivation may occur as a later event in tumor evolution in some cases.

### Hereditary Neuroblastoma

Although neuroblastoma usually occurs sporadically, 1% to 2% of patients report a family history of the disease. [40,41,42,43 and 44] This is similar to the other embryonal cancers of childhood, in which a subset of patients develop their cancers as a result of hereditary predisposition. Familial neuroblastoma is inherited in an autosomal dominant Mendelian fashion with incomplete penetrance. Affected children from these families differ from those with sporadic disease in that they are often diagnosed at an earlier age (usually infancy) and they frequently have multiple primary tumors. [41,43,44] These clinical characteristics are hallmarks of the “two-mutation” model for cancer predisposition first proposed for retinoblastoma. [45] Therefore, it appears likely that familial neuroblastoma occurs due to a germline mutation in one allele of a tumor suppressor gene (or genes). In addition, Knudson and Strong [43] proposed that germline mutations may account for initiation of tumorigenesis in up to 22% of sporadic neuroblastomas.

There is remarkable heterogeneity among patients with familial neuroblastoma. Within individual families, the disease can vary from asymptomatic ganglioneuroma or spontaneously regressing neuroblastoma to rapidly progressive and fatal disease. [46] Thus, the timing of inactivation of the second tumor suppressor gene allele and additional mutations are postulated to confer the ultimate clinical phenotype. The clinical heterogeneity of familial neuroblastoma may partially explain its rarity, because some tumors remain occult or regress and thus are never detected. In addition, others result in death before reproductive age, so the germline mutations are never passed on.

Many candidates for the hereditary neuroblastoma gene or locus have been proposed, including several regions of deletion containing putative tumor suppressor loci. Linkage analysis has excluded each of these candidate regions, including the distal short arm of chromosome 1. [40,41,47] However, a recent genome-wide search for linkage has identified the short arm of chromosome 16 as a likely site of a hereditary neuroblastoma predisposition gene. [48]

## CELLULAR AND MOLECULAR PATHOGENESIS

Considerable progress has been made in the past decade in understanding human neuroblastoma at a cellular and molecular level. [49,50,51,52,53 and 54] These studies have contributed to better methods of tumor diagnosis and subclassification, and they provide information that is useful in predicting clinical behavior. In addition to providing insights into mechanisms of malignant transformation and progression, these studies may identify critical genes, proteins, and pathways that may serve as targets for future therapy. In turn, these novel approaches to treatment may prove to be more effective and less toxic than current therapeutic modalities.

### Embryology

Several studies have demonstrated that microscopic neuroblastic nodules occur uniformly in the adrenal glands of all fetuses studied. [55,56] These nodules peaked between 17 and 20 weeks of gestation and gradually regressed by the time of birth or shortly after. Previously, Beckwith and Perrin [57] reported that microscopic

neuroblastic nodules, resembling “neuroblastoma *in situ*,” were found frequently in infants younger than 3 months who died of other causes. This finding was interpreted initially to indicate that neuroblastomas develop much more often than they are detected clinically and that the tumor regresses spontaneously in the majority of cases. However, these neuroblastic nodules most likely represent the remnants of normal fetal adrenal development. [55,56] Nevertheless, these neuroblastic cell rests likely represent the cells from which adrenal neuroblastomas develop.

It is unlikely that the microscopic neuroblastic nodules described above would be detected clinically, nor would they be detected by screening infants for neuroblastoma by measuring urinary catecholamine metabolites (see below). The concept of *in situ* neuroblastoma has been used to support the argument that the number of neuroblastomas that arise and regress spontaneously is many times the number detected clinically. Indeed, there are a number of well-documented cases in infants with neuroblastoma that have had complete regression of their tumor. [58,59 and 60] Mass screening studies in Japan, Quebec, and elsewhere, however, give a more realistic estimate of the number of neuroblastomas that regress without treatment. [61,62 and 63] It appears that approximately half of all neuroblastomas that reach a size that would be detectable by screening actually regress without specific therapy, whereas an equivalent number are detected clinically in unscreened populations.

**Neuronal Differentiation**

Neuroblastoma cells are derived from postganglionic sympathetic neuroblasts, and they frequently exhibit features of neuronal differentiation. Indeed, neuroblastomas may show spontaneous or induced differentiation to ganglioneuroblastoma or ganglioneuroma, so the malignant transformation of these cells may result in part from a failure to respond fully to the normal signals to undergo morphologic differentiation. The factors responsible for regulating normal differentiation are not understood completely at present, but they probably involve one or several neurotrophin receptor pathways that signal the cell to differentiate. One of the important pathways appears to be NGF and its receptor. Indeed, this pathway is representative of a family of homologous neurotrophins and receptors that may play important roles in regulating the survival, growth, and differentiation of sympathetic neuroblasts. Additional neurotrophic factors and receptors have been described recently, [64,65 and 66] so there is reason for optimism that the differentiation process may become understood better. This in turn may lead to better means of tumor classification or of treatment aimed at inducing differentiation or regression.

**Neurotrophin Receptors**

Neurotrophic factors and their receptors have been implicated in the pathogenesis of neuroblastoma, but their *precise* role has been unclear. Indeed, most neuroblastoma cell lines are neither dependent on nor responsive to the presence of NGF *in vitro*. Previous studies have demonstrated defects of expression and function of the NGF receptor in neuroblastoma cell lines, [67,68] but the role of the NGF receptor pathway in the pathogenesis of neuroblastomas has been uncertain.

NGF is a member of a family of homologous neurotrophins that includes BDNF, neurotrophin-3, and neurotrophin-4/5. [69] Recently, three TRK genes encoding receptors for the neurotrophic factors of the NGF family have been cloned. The genes *TRKA*, *TRKB*, and *TRKC* encode the primary receptors for NGF, BDNF and neurotropin-3, respectively. [69] The primary receptor for neurotropin-4/5 is unknown, but it appears to function through *TRKB*.

Nakagawara and colleagues [70,71] demonstrated that *TRKA* expression was inversely correlated with *MYCN* amplification. Indeed, high *TRKA* expression was associated with a biologically and clinically favorable group of patients (age younger than 1 year; stage 1, 2, and 4S) and with a very good outcome. [71,72,73,74,75 and 76] The combined assessment of *MYCN* copy number and *TRKA* expression provided additional prognostic information over either variable alone. Furthermore, studies have demonstrated that primary neuroblastoma cells with high *TRKA* expression differentiate in the presence of NGF *in vitro*, whereas the same cells die in the absence of NGF. [71] Thus, the NGF/*TRKA* pathway may explain the propensity for some neuroblastomas to differentiate or to regress spontaneously.

The studies of *TRKB* and *TRKC* expression in neuroblastomas are more limited. *TRKB* was expressed in about one-third of the neuroblastomas tested, and its cognate ligand BDNF was expressed in two-thirds of the tumors. [77] The truncated form of *TRKB*, lacking the tyrosine kinase domain, was expressed predominantly in more differentiated tumors (ganglioneuroblastomas and ganglioneuromas), whereas the full-length *TRKB* transcript was expressed in tumors with *MYCN* amplification. [77] Thus, the *TRKB*/BDNF pathway may serve as an autocrine or paracrine pathway to promote survival in *MYCN*-amplified tumors. In contrast, *TRKC* expression was found in approximately 25% of neuroblastomas tested, and its pattern of expression resembles that of *TRKA*. [78,79] Because all tumors with *TRKC* expression also had high *TRKA* expression, it did not add additional prognostic significance. However, it may represent an alternate or additional pathway for neuronal differentiation in these tumors.

**Neuropeptides**

Two other markers of neuronal differentiation sometimes associated with neuroblastomas are chromogranin A and neuropeptide Y. Chromogranin A is an acidic protein that is a component of neurosecretory granules of neuroendocrine cells, tissues, and tumors, and it is developmentally regulated. [80,81 and 82] It is present in the serum of patients with neuroblastoma, so it may serve as a sensitive and specific serum marker for disease activity and response to treatment. [83,84] Neuropeptide Y is another neurosecretory protein whose expression is developmentally regulated and restricted to the nervous system. [85,86] These two proteins may be useful in characterizing neuroblastomas in terms of their developmental state of differentiation or patient monitoring. [87,88 and 89] SS and VIP represent additional peptide hormones that are associated with neuroblastoma. Expression of SS receptors on neuroblastoma cells is associated with more differentiated histology, lower stages of disease, and a favorable outcome. [90,91,92 and 93] Furthermore, SS analogs can be used for neuroblastoma imaging *in vivo* (see below). Although VIP is associated with a paraneoplastic syndrome that includes watery diarrhea and abdominal distension (see below), VIP expression is also associated with differentiated tumors, and it can also induce neuronal differentiation in neuroblastoma cells in culture. [94,95] The role that these two proteins play in regulating growth or differentiation of sympathetic neurons during normal development is unclear.

**DNA Index**

Flow cytometric analysis of DNA content is a simple and semiautomated way of measuring total cell DNA content, which correlates well with modal chromosome number. Studies by Look and colleagues [96,97] and others have demonstrated that determination of the DI of neuroblastomas from infants provides important information that can be predictive of response to *therapy* as well as outcome. Interestingly, infants with tumors that have a “hyperdiploid” DNA content (DI greater than 1) are more likely to have lower stages of disease and to respond to initial therapy, whereas those with a “diploid” DNA content (DI = 1) more likely have advanced stages of disease and do not respond to this combination. [96,97] Although this analysis cannot detect specific chromosome rearrangements, such as deletions, translocations, or even gene amplification, it is a relatively simple test that correlates with biologic behavior, at least in this age group. The favorable prognostic association of hyperdiploidy appears restricted to infants, however, particularly those with advanced stages of disease. This is likely because whole chromosome gains account for the hyperdiploidy in this age group. [97] Hyperdiploid tumors in older children are more likely to have structural changes, and this karyotypic pattern is not associated with a favorable outcome. [97,98]

**Cytogenetic Characterization**

Neuroblastomas are characterized cytogenetically by recurrent deletions of 1p, 11q, and other sites, as well as unbalanced gain of 17q. The former abnormalities are thought to represent loss of tumor suppressor genes at these sites, whereas unbalanced 17q gain may represent a gain of function of a gene or genes on this chromosomal region. In addition, cytogenetic manifestations of gene amplification, such as dmins or HSRs are found in a subset of neuroblastomas. [99,100,101,102 and 103] Although the majority of tumors that have been karyotyped are in the diploid range, a substantial number from patients with lower stages of disease are hyperdiploid or near triploid. The modal karyotype number has been shown to have prognostic value, but the distinction between patterns of genetic change (whole chromosome gains versus structural changes) may be particularly important. [104,105 and 106]

***MYCN* Amplification and Expression**

Extrachromosomal dmins and chromosomally integrated HSRs are cytogenetic manifestations of gene amplification, but the presence of these findings per se does not indicate which gene is amplified. For neuroblastomas, the region amplified is virtually always derived from the distal short arm of chromosome 2, and it contains the proto-oncogene *MYCN* (also known as N-*myc*). Brodeur, Seeger and colleagues [107,108] have demonstrated that *MYCN* amplification occurs in approximately 25% of primary neuroblastomas from untreated patients (Fig. 31-2A); and amplification is associated predominantly with advanced stages of disease, rapid tumor progression, and a poor prognosis. Amplification is found in 5% to 10% of patients with low stages of disease and stage 4S and 30% to 40% of advanced disease

patients (Table 31-1).[24,101,102 and 103,109] *MYCN* amplification is almost always present at the time of diagnosis if it is going to occur, [107] so it appears to be an intrinsic biologic property of a subset of very aggressive tumors that frequently have a poor outcome.

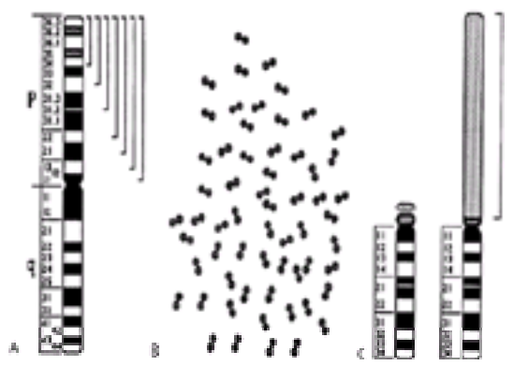


**FIGURE 31-2.** Common cytogenetic abnormalities in human neuroblastomas. Shown are diagrammatic representations of the three most common cytogenetic abnormalities seen in human neuroblastomas. **A:** Deletions of the short arm of chromosome 1. The brackets indicate that the region deleted in different tumors is variable in terms of its proximal breakpoint, but the distal short arm appears to be deleted in all cases, resulting in partial 1p monosomy. **B:** Extrachromosomal double minutes (dmins). Dmins are seen in approximately 30% of primary neuroblastomas and are a cytogenetic manifestation of gene amplification. **C:** A representative homogeneously staining region (HSR) on the short arm of chromosome 13 is shown in this example. HSRs are a cytogenetic manifestation of gene amplification in which the amplified sequences are chromosomally integrated. (From Brodeur GM. Neuroblastoma—clinical applications of molecular parameters. Brain Pathol 1990;1:45, with permission.)

| Stage at diagnosis | *MYCN* amplification (%) | 3-yr survival (%) |
|---|---|---|
| Benign ganglioneuromas | 0/64 (0) | 100 |
| Low stages (1, 2) | 31/772 (4) | 90 |
| Stage 4S | 15/190 (8) | 80 |
| Advanced stages | 612/1,974 (31) | 40 |
| Total | 658/3,000 (22) | 55 |

From Brodeur GM. Clinical and biological aspects of neuroblastoma. In: Vogelstein B, Kinzler KW, eds. The genetic basis of human cancer. New York: McGraw-Hill, 1998;691–711; Brodeur, GM. Molecular basis for heterogeneity in human neuroblastomas. Eur J Cancer 1995;31A:505–510; Brodeur GM, Maris JM, Yamishiro DJ, et al. Biology and genetics of human neuroblastomas. J Pediatr Hematol/Oncol 1997;19:93–101; and Brodeur GM, Ambros PF. Genetic and biological markers of prognosis in neuroblastoma. In: Brodeur GM, Sawada T, Tsuchida Y, et al., eds. Neuroblastoma. Amsterdam: Elsevier Science, 2000;355–369, with permission.

**TABLE 31-1. CORRELATION OF MYCN AMPLIFICATION WITH ADVANCED STAGE**

The genomic region amplified with *MYCN* is quite large, usually on the order of 500 to 1,000 kb. Therefore, it has been postulated that additional genes located near *MYCN* may contribute to the ultimate tumor phenotype when co-amplified. High-resolution restriction mapping of the *MYCN* locus has shown that a 130-kb core domain is the consistent target of amplification.[111] To date, no other gene besides *MYCN* has been identified in this core domain.[112] However, the RNA helicase gene *DDX1* maps within 300 kb 5' of *MYCN* and is co-amplified in approximately 40% to 50% of neuroblastomas with *MYCN* amplification.[113] *DDX1* can transform NIH-3T3 cells and allow for establishment of sarcomatous primary tumors in immunodeficient mice.[114] Nevertheless, *DDX1* amplification has not been identified in the absence of *MYCN* amplification. Thus, *MYCN* is primarily responsible for the aggressive nature of the neuroblastomas with amplification at the 2p24.1 locus, but *DDX1* may contribute to the highly malignant nature of some *MYCN*-amplified tumors.

In general, there is a correlation between *MYCN* copy number and expression, and tumors with amplification usually express *MYCN* at much higher levels than those seen in tumors without amplification.[70] Thus, *MYCN* overexpression in the context of amplification consistently identifies a subset of neuroblastomas with highly malignant behavior. However, it is controversial whether overexpression of *MYCN* messenger RNA or MycN protein has prognostic significance in nonamplified tumors.[115,116 and 117] Some neuroblastoma cell lines express high levels of *MYCN* messenger RNA or MycN protein without gene amplification.[116,118] This may be due to alterations in normal protein degradative pathways[119] rather than loss of *MYCN* transcriptional autoregulation.[120] Some studies have suggested that MycN expression correlates inversely with survival probability,[121] whereas others have found no such correlation.[122] Further studies in a larger cohort of consistently treated patients with standardized methods will be necessary to determine whether quantitative assessment of MycN expression in tumors lacking *MYCN* amplification provides additional prognostic information.

### Amplification and Expression of Other Oncogenes

No other human oncogene has been shown to be consistently amplified, mutated, or overexpressed in neuroblastoma. Mutations of members of the *RAS* gene family are common in many other types of cancer, but the role of *RAS* genes in neuroblastomas is unclear. *NRAS* was originally identified as a transforming gene in a neuroblastoma cell line, and *RAS* can cooperate with *MYCN* to transform embryonic fibroblasts.[123,124] In addition, targeting of *HRAS* overexpression to the neuroectoderm of mice causes ganglioneuromas and occasional neuroblastomas,[125] but *HRAS* overexpression has been associated with a favorable outcome in neuroblastomas.[76,126] In any case, activating mutations of any members of the *RAS* gene family are rarely observed in primary neuroblastomas.[127,128 and 129] Co-amplification of *MYCL* or *MDM2* has been observed with *MYCN* in a few neuroblastoma cell lines,[130,131 and 132] and recent CGH studies have identified other novel regions of amplification at 2p13-15, 2p23, 3q24-26, 4q33-35, and 6p11-22 in occasional tumors.[133,134] However, the biologic significance of these observations has not yet been defined.

### Unbalanced Gain of 17q

Recurrent abnormalities of the long arm of chromosome 17 were originally identified by analysis of Giemsa-banded karyotypes derived from neuroblastoma primary tumors and cell lines.[135] It has recently become apparent that unbalanced gain of distal 17q material is perhaps the most common genetic abnormality in primary neuroblastomas (Fig. 31-3). Unbalanced 1;17 translocations occur frequently in primary neuroblastomas [136,137] and often result in loss of distal 1p with concomitant gain of distal 17q material.[138] However, the 17q translocation breakpoints are heterogeneous and often involve other partner chromosomes, most notably 11q.[139] Bown and colleagues[140] demonstrated that 54% of 313 neuroblastomas analyzed at diagnosis had unbalanced 17q21qter gain. Unbalanced 17q gain is associated with adverse prognostic features and is present in the vast majority of neuroblastomas with *MYCN* amplification.[140,141,142 and 143] Thus, this genomic region is likely to harbor a gene that contributes to neuroblastoma tumorigenesis when present in increased copy number or overexpressed.

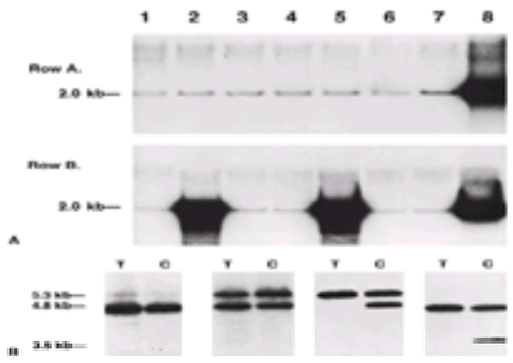



**FIGURE 31-3. A:** Southern blots showing *MYCN* amplification and 1p allelic loss. Lane 1 represents DNA from a normal lymphoblastoid cell line as a single-copy control, and lane 8 represents DNA from the NGP cell line, with 150 copies of *MYCN* per haploid genome. (*Row A*): Lanes 2 through 7 represent six neuroblastomas with a single copy of *MYCN* per haploid genome. (*Row B*): Lanes 2 and 5 show examples of tumors with *MYCN* amplification, whereas the other tumors have the normal single-copy signal. (From Brodeur GM. Neuroblastoma—clinical applications of molecular parameters. Brain Pathol 1990;1:45, with permission.) **B:** 1p allelic loss. Southern hybridization with a polymorphic probe (D1S57) to normal and tumor DNA after restriction enzyme digestion. The first panel shows a case in which polymorphism was not seen in the constitutional DNA (C), so the case is uninformative with respect to allelic loss or LOH in the tumor DNA (T). The second panel shows a case in which polymorphism was seen in the constitutional DNA, so it was informative, but no LOH was seen in the tumor. The last two panels show cases in which the constitutional DNA was informative, and LOH was detected in the tumor, as demonstrated by the absence of the lower band in both cases. (From Brodeur GM, Fong CT. Molecular biology and genetics of human neuroblastoma. Cancer Genetics Cytogenet 1980;41:153–174, with permission.)

## Chromosome Deletion or Allelic Loss

### *Chromosome 1*

Deletions of 1p are found in 70% to 80% of the near-diploid tumors that have been karyotyped. [99,104,106,135,144,145,146 and 147] Molecular genetic studies have confirmed these observations by documenting LOH in approximately 35% of primary tumors (Fig. 31-2B; Fig. 31-3) and in the vast majority of neuroblastomas with *MYCN* amplification.[148,149,150,151,152,153 and 154] Addition of an intact human chromosome 1p to a 1p-deleted neuroblastoma cell line induced cellular differentiation or death. [155] It therefore appears likely that a neuroblastoma suppressor gene is located on the short arm of chromosome 1 and that this gene is inactivated in at least one-third of primary neuroblastomas. However, the majority of 1p deletions are large and the proximal breakpoints heterogeneous, [149,154,156,157,158 and 159] suggesting that distal 1p may contain more than one suppressor gene critical for malignant transformation or progression.

### *Chromosome 11*

Several lines of evidence suggest that a neuroblastoma suppressor gene is located on the long arm of chromosome 11. Chromosome 11q deletions have been noted in approximately 20% of reported neuroblastoma karyotypes (Fig. 31-3),[147] and transfer of an intact chromosome 11 into a neuroblastoma cell line induced differentiation.[155] Constitutional rearrangements of chromosome 11q have also been observed in four neuroblastoma patients. [160] Molecular genetic studies have demonstrated loss of 11q in more than one-third of primary tumors[133,134,143,151,161,162] Guo and colleagues[162] mapped a common region of deletion to 11q23, indicating that this is the most likely location of an 11q neuroblastoma suppressor gene. In contrast to 1p LOH, there was a striking *inverse* correlation of 11q LOH with *MYCN* amplification.

### *Chromosome 14*

Deletion of the long arm of chromosome 14 is also a common abnormality in neuroblastomas (Fig. 31-3).[151,161,163,164] Unlike for chromosomes 1 and 11, there are only a few reports of cytogenetically visible deletions or rearrangements involving 14q. [145,165] This may indicate that deletions of chromosome 14 are too small to be identified easily by conventional cytogenetic analysis or that the mechanism of LOH involves homologous mitotic recombination. A recent study of 372 primary neuroblastomas with markers evenly spaced along 14q showed LOH in 22%, with a common region of deletion within 14q23qter. [166] LOH for 14q was highly correlated with 11q LOH and *inversely* related to 1p36 LOH and *MYCN* amplification. Furthermore, data from another group have suggested that there may be two distinct regions of allelic loss on 14q, [167] so there may be more than one suppressor gene on this chromosome arm.

### *Deletions of Other Chromosomal Regions*

There are other regions of the genome that are frequently deleted in neuroblastoma DNAs, suggesting the existence of additional tumor suppressor genes. There have been reports of LOH or allelic imbalance by CGH at chromosome arms 3p,[168] 4p,[169] 9p,[170,171] and 18q,[172] but these appear less common than 1p or 11q LOH. The genes that are targets for these apparent nonrandom deletions are unknown.

### *Alterations of Known Tumor Suppressor Genes*

No novel tumor suppressor genes have been isolated to date, and there is currently no evidence for consistent mutation in any known tumor suppressor genes. *TP53* is the most frequently mutated gene in human cancer, but it is rarely inactivated by deletion or mutation in neuroblastomas obtained at diagnosis. [173,174,175 and 176] Moll and colleagues[177] have reported aberrant cytoplasmic localization of the p53 protein in neuroblastoma and found evidence for dysregulated $G_1$/S checkpoint control. However, others have shown that DNA damage to neuroblastoma cells causes normal translocation of wild-type p53 to the nucleus and induction of p21. [178] *CDKN2* encodes p16, another cell cycle control protein commonly inactivated in human cancers, but mutations and deletions have rarely been found in neuroblastomas. [179,180] The *DCC* and *DPC4* genes are located at 18q, a region that is frequently deleted in primary neuroblastomas. Although expression of *DCC* may be altered in some cell lines and tumors, no inactivating mutations of *DCC* or *DPC4* have been identified.[172,173,174,175,176,177,178,179,180 and 181]

## Genetic Model of Neuroblastoma Development

In summary, there is increasing evidence for two or three genetic subsets of neuroblastomas that are highly predictive of clinical behavior. One recently proposed classification takes into account abnormalities of 1p, *MYCN* copy number, and assessment of DNA content, and distinct genetic subsets of neuroblastomas can be identified (Fig. 31-4, Table 31-2).[24,103,109] The first group (type 1) is characterized by mitotic dysfunction leading to a hyperdiploid or near-triploid modal karyotype, with few if any cytogenetic rearrangements. These tumors lack specific genetic changes such as *MYCN* amplification or 1p LOH, and they have high *TRKA* expression. These patients are generally younger than 1 year, with localized disease and a very good prognosis. Most of the infants detected by the neuroblastoma screening studies fall into this category. The second group is characterized by gross chromosomal aberrations, and they generally have a near-diploid karyotype. No consistent abnormality has been identified to date, but 17q gain is common. Within this type, two subsets can be distinguished. One subset (type 2) is characterized by 11q deletion, 14q deletion, or other changes, but it lacks *MYCN* amplification and generally lacks 1p LOH. Patients with these tumors are generally older, with more advanced stages of disease that is slowly progressive but often fatal. The other subset (type 3) is characterized by amplification of *MYCN*, usually with 1p36 LOH and high *TRKB* expression. These patients are generally between 1 and 5 years of age, with advanced stages of disease that is rapidly progressive and frequently fatal. It is unknown if a tumor from one type ever converts to a less favorable type, but current evidence suggests that they are genetically distinct.

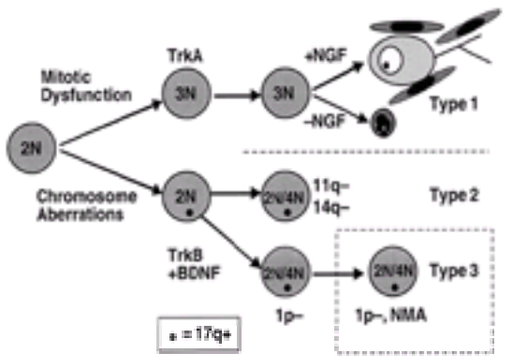



**FIGURE 31-4.** Genetic model of neuroblastoma development. According to this model, all neuroblastomas have a common precursor (NB) and may have a common mutation (the one responsible for familial neuroblastoma). However, a commitment is made to develop into one of three major types. The first type is characterized by mitotic dysfunction, leading to a hyperdiploid or near-triploid modal karyotype (3N) with whole chromosome gains but few if any structural cytogenetic rearrangements. These tumors usually express high levels of NGF receptor *TRKA*, so they are prone to either differentiation or apoptosis, depending on the presence or absence of NGF in their microenvironment. The second type generally has a near-diploid (2N) or near-tetraploid karyotype but is characterized by gross chromosomal aberrations. No consistent abnormality has been identified to date, but 17q gain is common, and allelic loss of 1p, 11q, or 14q is seen frequently. The third type is related to the second but is characterized by *MYCN* amplification. These tumors frequently have allelic loss of 1p. The latter tumors frequently express *TRKB* plus BDNF, probably representing an autocrine survival pathway. Thus, neuroblastoma represents fundamentally two major types and three subtypes, but they may all arise from a common precursor cell. NMA, *MYCN* ampification.

| Feature | Type 1 | Type 2 | Type 3 |
|---|---|---|---|
| *MYCN* | Normal | Normal | Amplified |
| DNA ploidy | Hyperdiploid<br>Near triploid | Near diploid<br>Near tetraploid | Near diploid<br>Near tetraploid |
| 11q gain | Rare | Common | Common |
| 11q, 14q LOH | Rare | Common | Rare |
| 1p LOH | Rare | Uncommon | Common |
| *TRKA* expression | High | Low or absent | Low or absent |
| *TRKB* expression | Truncated | Low or absent | High (full length) |
| *TRKC* expression | High | Low or absent | Low or absent |
| Age | Usually <1 yr | Usually >1 yr | Usually 1–5 yr |
| Stage | Usually 1, 2, 4S | Usually 3, 4 | Usually 3, 4 |
| Three-year survival (%) | 95 | Approximately 50 | Approximately 25 |

From Brodeur GM. Clinical and biological aspects of neuroblastoma. In: Vogelstein B, Kinzler KW, eds. The genetic basis of human cancer. New York: McGraw-Hill, 1998:691–711; Brodeur GM. Molecular basis for heterogeneity in human neuroblastomas. Eur J Cancer 1995;31A:505–510; Brodeur GM, Maris JM, Yamashiro DJ, et al. Biology and genetics of human neuroblastomas. J Pediatr Hematol Oncol 1997;19:93–101; and Brodeur GM, Ambros IV. Genetic and biological markers of prognosis in neuroblastoma. In: Brodeur GM, Sawada T, Tsuchida Y, et al., eds. Neuroblastoma. Amsterdam: Elsevier Science, 2000:315–361; with permission.

**TABLE 31-2. GENETIC/CLINICAL SUBTYPES OF NEUROBLASTOMA**

## PATHOLOGY

Neuroblastoma is one of the “small, round blue cell” neoplasms of childhood; also included are Ewing's sarcoma, non-Hodgkin lymphoma, primitive neuroectodermal tumors (or neuroepitheliomas), and undifferentiated soft tissue sarcomas (including rhabdomyosarcoma). Neuroblastomas presumably arise from primitive, pluripotential sympathetic cells (sympathogonia), which are derived from the neural crest. The degree and type of differentiation, and the location of the sympathogonia after migrating from the neural crest, generate the different normal tissues of the sympathetic nervous system, including spinal sympathetic ganglia and adrenal chromaffin cells. The histologic subtypes of the neuroblastic tumors appear to correlate with the normal differentiation patterns of the sympathetic nervous system.

The three classic histopathologic patterns of neuroblastoma, ganglioneuroblastoma, and ganglioneuroma reflect a spectrum of maturation and differentiation ( Fig. 31-5). The typical neuroblastoma is composed of small but uniformly sized cells containing dense, hyperchromatic nuclei and scant cytoplasm. The presence of neuritic processes, or neuropil, is a pathognomonic feature of all but the most primitive neuroblastomas. The Homer-Wright pseudorosette ( Fig. 31-6), another diagnostic feature of neuroblastoma seen in 15% to 50% of cases,[182] is composed of neuroblasts surrounding areas of eosinophilic neuropil.

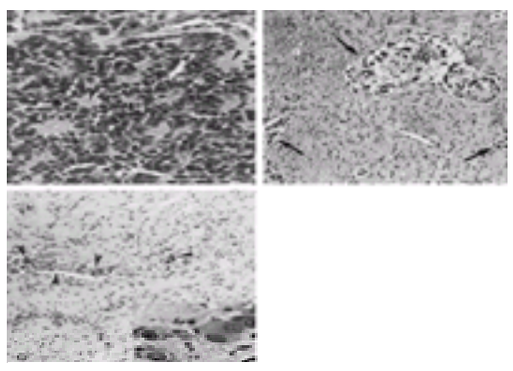


**FIGURE 31-5.** The principal histopathologic subtypes of neuroblastoma. **A:** Neuroblastoma, with monotonous arrays of hyperchromatic cells with scant cytoplasm, rosettes, and minimal neuropil (*arrowheads*). (Hematoxylin and eosin, ×132.) **B:** Ganglioneuroblastoma with rests of neuroblasts (*arrows*) within Schwannian stroma. (Hematoxylin and eosin, ×33.) **C:** Ganglioneuroma demonstrating mature ganglion cells (*arrowheads and inset*) with Schwannian stroma. (Hematoxylin and eosin, ×33, inset ×198.) (Courtesy David Kelly, University of Alabama at Birmingham and Vijay Joshi, East Carolina University.)

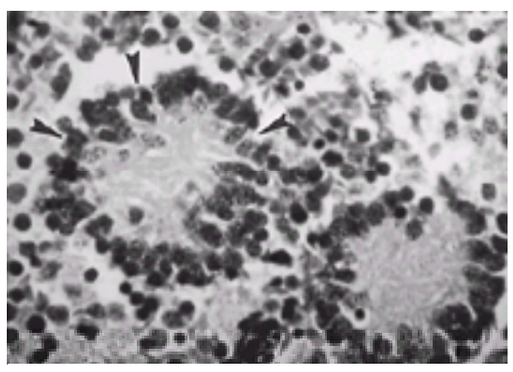


**FIGURE 31-6.** Homer-Wright pseudorosettes are typically rings of neuroblasts ( *arrowheads*) surrounding a central core of eosinophilic neuropil. (Hematoxylin and eosin, ×198). (Courtesy David Kelly, University of Alabama at Birmingham.)

The fully differentiated and benign counterpart of neuroblastoma is a *ganglioneuroma.* It is composed of mature ganglion cells, neuropil, and Schwannian cells. *Ganglioneuroblastoma* defines a heterogeneous group of tumors with histopathologic features spanning the extremes of maturation represented by neuroblastoma and ganglioneuroma. Histopathologic characteristics range from a predominance of neuroblastic elements with rare maturing cells to those neoplasms comprised almost exclusively of ganglioneuroma containing occasional rests of neuroblasts. Some use the term *maturing neuroblastoma* for tumors that contain less than 50% maturing or mature ganglion cells, and *ganglioneuroblastoma* for tumors with more extensive maturation. Ganglioneuroblastomas may be focal or diffuse, depending on the gross pattern seen, but diffuse ganglioneuroblastoma is associated with less aggressive behavior. Because tumor viability and histopathologic features may vary within a single tumor, multiple sections, particularly from regions with different gross appearance, should be examined to categorize these tumors accurately.

Distinguishing neuroblastoma from other small, round blue cell tumors of childhood often requires techniques beyond hematoxylin and eosin staining and light microscopy. Immunohistochemistry (e.g., immunoperoxidase techniques) is a helpful adjunct to light microscopy. Neuroblastomas will stain with monoclonal antibodies recognizing neurofilament proteins, synaptophysin, and NSE.[183,184] Electron microscopy typically demonstrates dense core, membrane-bound neurosecretory granules as well as microfilaments and parallel arrays of microtubules within the neuropil ( Fig. 31-7)[185,186] but is seldom used for diagnostic purposes.

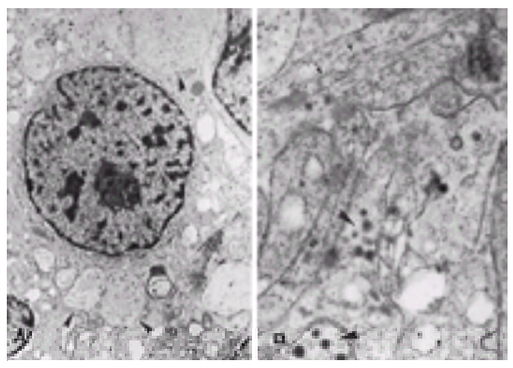


**FIGURE 31-7.** Electron micrograph of a neuroblast. **A:** In transverse cross section are numerous primitive dendritic processes of neuroblasts ( *arrowheads*) surrounding the cell body of a neuroblast. The neuroblast has a high nuclear to cytoplasmic ratio and relatively few organelles. (Uranyl acetate and lead citrate, ×9,500.) **B:** Dendritic processes (neurites) of varying sizes lie in close juxtaposition. Some processes contain many microtubules ( *small arrowheads*), whereas others display small clusters of dense core neurosecretory granules ( *large arrowheads*). (Uranyl acetate and lead citrate, ×40,000.) (Courtesy David Kelly, University of Alabama at Birmingham.)

The prognostic classification of neuroblastoma based on histopathologic features has been attempted by several investigators. [187,188,189] and [190] The most widely used histopathologic classification system was developed by Shimada and colleagues [189] and is formulated around patient age and the following histologic features: the presence or absence of Schwannian stroma, the degree of differentiation, and the MKI. A retrospective evaluation of the Shimada method in 295 CCG patients identified favorable and unfavorable patient subsets. When compared to other clinical features, these histologic patterns were independently predictive of outcome, and stage was prognostically less important than histologic grade.[189,191,192]

In an attempt to simplify the Shimada system yet maintain the predictive power of histopathologic features, Joshi and colleagues [193,194] conducted a retrospective analysis of pathologic data collected in the POG from 211 patients with neuroblastoma. The presence of calcification and a low mitotic rate (less than or equal to ten mitoses per ten high-power fields) predicted a favorable outlook, independent of stage or age. The Joshi method provided greater simplicity in determining the histologic features and prognostic categories but did not demonstrate the same prognostic power as the Shimada system. Therefore, Shimada, Joshi and other noted pediatric pathologists have developed an International Neuroblastoma Pathology Classification that combines the best features of these two systems (see below).[195,196] This classification is based primarily on the Shimada system but incorporates other features that should permit improved concordance among pathologists using this system around the world.

## CLINICAL PRESENTATION AND PATTERN OF SPREAD

Because neuroblastoma can arise from any site along the sympathetic nervous system chain, the locations of primary tumors at the time of diagnosis are varied and change with age. Most primary tumors occur within the abdomen (65%), although the frequency of adrenal tumors is slightly higher in children (40%) compared to infants (25%). Infants also have more thoracic and cervical primary tumors. In approximately 1% of patients, a primary tumor cannot be found. The majority of children with neuroblastoma is diagnosed by age 5 years, and it is rare after age 10 years ( Fig. 31-1).

Metastatic extension of neuroblastoma occurs in lymphatic and hematogenous patterns. Regional lymph node metastases are noted in 35% of patients with apparently localized tumors. Spread of tumor to lymph nodes outside the cavity of origin is considered to be disseminated disease. However, these children may have a better outlook if there is no other metastatic disease noted.[197] Hematogenous spread occurs most frequently to bone marrow, bone, liver, and skin. Rarely, disease may spread to lung and brain parenchyma, usually as a manifestation of relapsing or end-stage disease. The proportion of patients presenting with localized, regional, or metastatic disease is age dependent. In 910 consecutive CCG patients, the prevalence of INSS stage 3 and 4 disease was 41% in infants compared to 80% in older children (Table 31-3).

| | Age at diagnosis | | |
|---|---|---|---|
| INSS stage | <1 yr (%) | ≥1 yr (%) | Total (%) |
| 1 | 95 (27)[a] | 72 (13) | 167 (18) |
| 2A | 19 (5) | 13 (2) | 32 (4) |
| 2B | 33 (9) | 29 (5) | 62 (7) |
| 3 | 61 (17) | 113 (20) | 174 (19) |
| 4 | 82 (24) | 334 (60) | 416 (46) |
| 4S | 59 (17) | 0 (0) | 59 (6) |
| Total | 349 | 561 | 910 |

[a]No. patients (%).
Courtesy Daniel Stram and Robert Gerbing, Children's Cancer Group Statistical Office, Arcadia, California.

**TABLE 31-3. EXTENT OF DISEASE AT DIAGNOSIS ACCORDING TO AGE**

The signs and symptoms of neuroblastoma reflect the location of primary, regional, and metastatic disease. Abdominal disease results in complaints of fullness, discomfort, and, rarely, obstruction. Physical examination commonly reveals a fixed, hard abdominal mass. When primary tumors arise from the organ of Zuckerkandl, bladder and bowel symptoms may occur due to compression. Massive involvement of the liver in metastatic disease is particularly frequent in infants (e.g., stage 4S) and may result in respiratory compromise. Occasionally, the size of primary or metastatic abdominal tumors can result in compression of venous and lymphatic drainage from the lower extremities leading to scrotal and lower extremity edema ( Fig. 31-8A). Rarely, patients will experience renin-mediated hypertension due to compromise of renal vasculature.[198,199] Because epinephrine is rarely released from most neuroblastomas, hypertension, tachycardia, flushing, and sweating are uncommon symptoms.

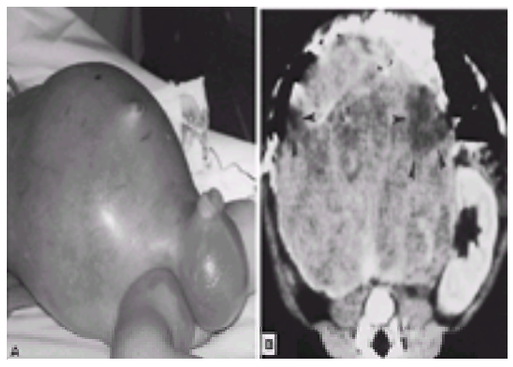


**FIGURE 31-8.** Clinical findings in abdominal neuroblastoma. **A:** Dramatic abdominal distention and scrotal edema in a 2-month-old patient. **B:** Computed tomography of abdomen of patient in **(A)** reveals a large suprarenal mass on the right with hemorrhage (*large arrowheads*) and calcification (*small arrowheads*). (Courtesy Walter Cain and Gregory Odrezin, University of Alabama at Birmingham.)

Sudden and dramatic enlargement of abdominal tumors with resultant increased abdominal distention and discomfort can result from spontaneous hemorrhage into the tumor (Fig. 31-8B). Primary thoracic tumors often are diagnosed coincidentally when chest radiographs are obtained to evaluate patients for trauma or infectious disease. High thoracic and cervical masses can be associated with Horner's syndrome, which consists of unilateral ptosis, myosis, and anhydrosis ( Fig. 31-9). Occasionally, large thoracic tumors are associated with mechanical obstruction and resultant superior vena cava syndrome. Cervical masses from primary or metastatic neuroblastoma may be confused with infection and are correctly diagnosed only at the time of attempted incision and drainage. Paraspinal tumors in the thoracic, abdominal, and pelvic regions may extend into the neural foramina of the vertebral bodies and result in symptoms related to compression of nerve roots and spinal cord. The range of symptomatology includes radicular pain and subacute or acute paraplegia as well as bladder or bowel dysfunction.

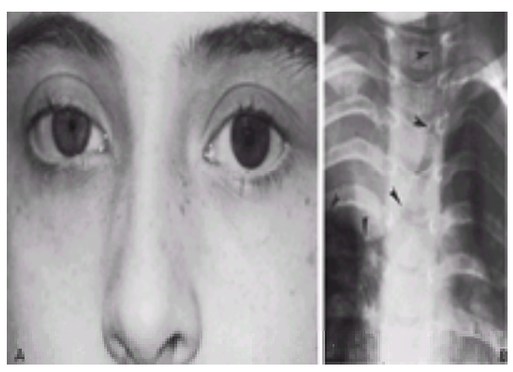


**FIGURE 31-9.** Horner's syndrome **(A)** with anisocoria secondary to a large, superior mediastinal mass and **(B)** causing tracheal deviation (*arrowheads*).

Several classical signs and symptoms have been associated with metastatic neuroblastoma. Proptosis and periorbital ecchymoses are frequent and result from tumor infiltration of periorbital bones ( Fig. 31-10). Widespread bone and bone marrow disease causes bone pain, which can lead to limping and irritability in a younger child. In addition, there may be bone marrow replacement and symptoms of bone marrow failure, such as anemia, bleeding, or increased risk of infection. Hematologic manifestations leading to bleeding or thrombosis have also been reported. [200,201 and 202] Skin involvement is seen almost exclusively in infants with INSS stage 4S tumors[203] and is characterized by variable numbers of nontender, bluish subcutaneous nodules. Constitutional symptoms associated with disseminated disease may include failure to thrive and fever, the latter observed most often in the presence of extensive bone metastases.

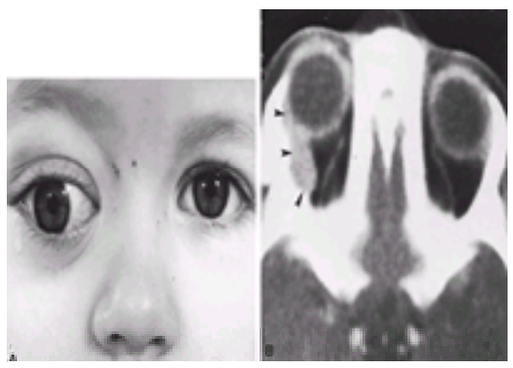


**FIGURE 31-10.** Proptosis on the right **(A)** in a 14-month-old patient who demonstrated on computed tomography scan **(B)** metastatic neuroblastoma in the retrobulbar space (*arrowheads*). (Courtesy Laura Bowman, St. Jude Children's Research Hospital, Memphis, TN.)

Although a minority of the overall patient population, adolescents and adults with neuroblastoma have been reported. [204,205,206 and 207] In general, the distribution of primary sites is similar to that seen in children, but the course of the disease is somewhat more indolent. [206,207] Also, neuroblastomas in these patients may be less sensitive to chemotherapy. Biologically, neuroblastomas from adolescents and adults fit genetic type 2 ( Table 31-2),[101,102,208] as they lack *MYCN* amplification but usually have near-diploid karyotypes with structural changes. [97] Tumors in older patients represent a special challenge, because even localized tumors can be recurrent and fatal over long periods.

**Paraneoplastic Syndromes**

Several unique paraneoplastic syndromes have been associated with both localized and disseminated neuroblastoma. Opsomyoclonus (myoclonic jerking and random eye movement) or cerebellar ataxia has been observed in up to 4% of patients. [209,210 and 211] Most children with this syndrome have a favorable outcome with respect to their tumor, although the outcome seems to correlate with the presence or absence of *MYCN* amplification.[212,213] The majority of these children appears to have long-term neurologic deficits, however, including cognitive and motor delays, language deficits, and behavioral abnormalities. [214,215] These are presumably due to antineural antibodies directed against the tumor that cross-react with neural cells in the cerebellum or elsewhere in the brain. [211,216,217 and 218] There is the suggestion that patients who received chemotherapy as part of their initial tumor management had fewer long-term side effects, suggesting that immunosuppression at a diagnosis may be beneficial.[216] In addition, most have improvement with adrenocorticotropic hormone or steroid therapy as well as tumor removal, although the majority has persistent or recurrent neurologic sequellae. [219,220 and 221] This indicates that one should anticipate long-term neurologic abnormalities, and early intervention may minimize these deficits.

Intractable secretory diarrhea associated with hypokalemia and dehydration is a manifestation of tumor secretion of VIP. [222,223 and 224] Most tumors secreting VIP are mature histologically (ganglioneuroblastomas or ganglioneuroma), and these patients almost always have a favorable outcome. [90] Surgical removal of the tumor

usually results in complete resolution of symptoms. Hypercalcemia has been reported also, [225] but it is uncommon and its origin is unknown.

### Neurologic Manifestations

In addition to the opsomyoclonus syndrome, there are other neurologic manifestations of neuroblastoma. Because of its origin next to the spinal column, neuroblastomas have a tendency to invade through neural foramina into the spinal canal and cause spinal cord compression. This situation can be a medical emergency (see Chapter 39), and there is controversy as to the optimum approach to managing spinal cord compression (see below). In addition to cord compression, neuroblastoma can disseminate to the central nervous system. This occurs most commonly by inward compression on the brain from cranial metastases, but it can occur also as meningeal involvement or as intracranial disease without adjacent parameningeal metastases [226,227,228,229,230 and 231] The route of spread may be hematogenous or via the cerebrospinal fluid. Although dissemination in the central nervous system is rarely seen at diagnosis, it may occur with progression or relapse and is regarded as a very ominous sign.

## METHODS OF DIAGNOSIS

Neuroblastomas generally arise in the adrenal medulla or along the sympathetic chain. To confirm this diagnosis, generally some histologic evidence is required that demonstrates neural origin or differentiation by light microscopy or immunohistology. Alternatively, because the bone marrow is frequently involved, some patients can be diagnosed with neuroblastoma based on the presence of "compatible" tumor cells involving the bone marrow accompanied by increased urinary catecholamine metabolites. In the past, some have used a compatible radiographic or scintigraphic appearance with increased urinary catecholamine metabolites. However, using this approach precludes the availability of tissue for critical diagnostic, prognostic, and research studies. Indeed, differences in diagnostic criteria for neuroblastoma have led to some difficulties in comparing studies from different institutions, but an international consensus on diagnostic criteria for neuroblastoma should avoid this problem (see Diagnostic Criteria).

### Catecholamine Metabolism

If sensitive techniques are used, increased urinary metabolites can be detected in 90% to 95% of neuroblastoma patients. [232,233 and 234] This provides a great advantage in confirming the diagnosis of neuroblastoma, as well as in following disease activity in those patients whose tumors are secretors. A diagrammatic representation of catecholamine synthesis and metabolism is depicted in Figure 31-11. Although the major pathways and products of catecholamine catabolism are shown, the actual pathways of intracellular and extracellular catecholamine breakdown are more complex.

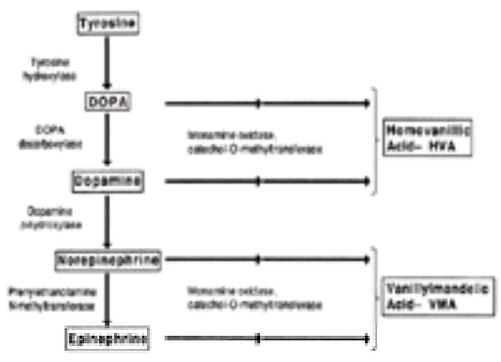



**FIGURE 31-11.** Pathway of catecholamine metabolism. Shown is a simplified diagram of catecholamine synthesis and metabolism. Homovanillic acid and vanillylmandelic acid are the urinary catecholamine metabolites usually measured. (From Brodeur GM. Neuroblastoma and other peripheral neuroectodermal tumors. In: Fernbach DJ, Vietti TJ, eds. Clinical pediatric oncology, 4th ed. St. Louis: Mosby, 1991;337, with permission.)

The precursor amino acids for catecholamine synthesis are phenylalanine and tyrosine. Phenylalanine is converted by phenylalanine hydroxylase to tyrosine. Tyrosine is then converted by tyrosine hydroxylase to DOPA, which is a catecholamine precursor. DOPA is converted by DOPA decarboxylase to the first catecholamine in the pathway, dopamine. Dopamine is converted by dopamine-b-hydroxylase to norepinephrine, which is then converted by phenylethanolamine-N-methyltransferase to epinephrine. Neuroblastoma cells lack this last enzyme, which is present in adrenal chromaffin cells and pheochromocytomas. The two enzymes primarily responsible for catecholamine catabolism are catechol-O-methyl transferase and monamine oxidase. DOPA and dopamine are converted primarily to HVA, whereas norepinephrine and epinephrine are converted primarily to VMA. Most laboratories involved in neuroblastoma diagnosis measure both urinary VMA and HVA.

### Diagnostic Criteria

An agreement on minimum criteria for establishing a diagnosis of neuroblastoma has been reached by an international group of conferees and corresponding participants, representing most of the major pediatric oncology groups throughout the world. [235,236] A diagnosis of neuroblastoma is established if

- An unequivocal pathological diagnosis is made from tumor tissue by light microscopy, with or without immunohistology, electron microscopy, or increased urine (or serum) catecholamines or metabolites; or
- Bone marrow aspirate or trephine biopsy contains unequivocal tumor cells (e.g., syncytia or immunocytologically positive clumps of cells), *and* increased urine or serum catecholamines or metabolites.

If histology is equivocal, and karyotypic abnormalities in tumor cells are characteristic of other tumors [e.g., t(11;22)], a diagnosis of neuroblastoma is excluded, whereas genetic features characteristic of neuroblastoma (e.g., *MYCN* amplification) would support this diagnosis.

At least two catecholamines and metabolites such as urine HVA and VMA (at a minimum) or serum dopamine or norepinephrine (assuming appropriate controls and standardization) must be measured. Levels must be greater than 3.0 SD above the mean for age to be considered increased. Normalizing urinary VMA and HVA excretion to the mg creatinine in the sample makes a timed collection unnecessary, and it avoids most false-negatives due to dilute urine on spot testing. Also, measurement of catecholamines and metabolites by sensitive techniques like high-pressure liquid chromatography, gas chromatography with mass spectroscopy, or specific enzyme-linked immunosorbent assays avoids the false-positives sometimes encountered with concentrated urine or with certain dietary components.

These criteria exclude the combination of a compatible radiographic or scintigraphic appearance and increased urinary catecholamine metabolites. Although allowing this definition may spare some patients an initial surgical procedure to confirm the diagnosis, patients with ganglioneuromas, pheochromocytomas, or other neural tumors potentially could receive inappropriate treatment. [237,238 and 239] In addition, biologic characterization of neuroblastoma cells is critically important for diagnosis or prognostication—for example, histopathology, *MYCN* gene copy number, tumor cell DI, or expression of *TRKA*. To perform the necessary biologic studies, it is critical to obtain tumor tissue as part of the initial diagnostic workup.

### Differential Diagnosis

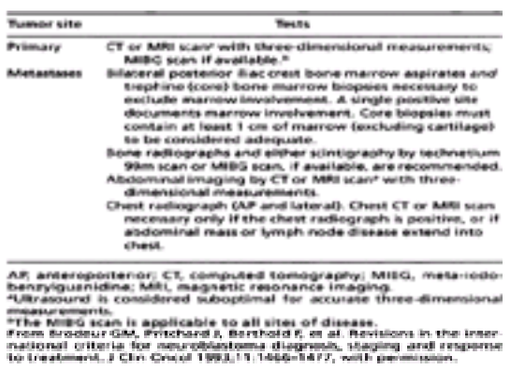
Due to the many potential clinical presentations, neuroblastoma may be confused with a variety of other neoplasms as well as non-neoplastic conditions. This is a problem particularly in the 5% to 10% of tumors that do not produce catecholamines [61,240,241] as well as the 1% of patients who do not have an obvious primary tumor. Alternatively, neuroblastoma should be considered in the differential diagnosis of a variety of non-neoplastic conditions or presenting symptoms. Clinically, patients with disseminated bone disease may resemble those with systemic infections or inflammatory diseases, such as osteomyelitis or rheumatoid arthritis. The VIP syndrome can be confused with infectious or inflammatory bowel disease, [222,224] and the opsoclonus-myoclonus and ataxia syndromes can resemble primary neurologic disease. [210,211,242] Neuroblastoma may be confused with calcified adrenal gland(s) following adrenal hemorrhage. [243,244] Patients with hepatomegaly must

have polycystic or storage diseases considered.

Histologically, neuroblastoma tissue from primary or metastatic sites may be quite undifferentiated and may be confused with other small, round blue cell tumors (see the section on pathology). Differential diagnosis of metastatic disease involving the marrow must include rhabdomyosarcoma, Ewing's sarcoma/neuroepithelioma/primitive neuroectodermal tumor, lymphoma, or leukemia (especially megakaryoblastic leukemia). However, a battery of monoclonal antibodies have been developed that allow reliable histopathologic diagnosis of these various disease entities. [186,245,246,247 and 248]

## CLINICAL AND LABORATORY EVALUATION

The recommended minimum testing to define the clinical stages is presented in Table 31-4. Certainly, the more tests that are performed, the greater the likelihood of finding disseminated disease. Uniformity with respect to minimum testing should improve the comparability of studies, and the tests recommended can be done in most medical centers. The conventional diagnostic imaging modalities include plain radiographs, bone scintigraphy, [249,250 and 251] ultrasound,[252] CT,[253,254] and MRI.[255,256] Plain radiographs can be useful to detect a calcified abdominal mass or posterior mediastinal mass. Both bone scintigraphy and skeletal survey should be used to detect cortical bone metastases.[250,257] Ultrasound and CT provide more information about abdominal disease, including lymph node enlargement and liver metastases, whereas thoracic CT or MRI are useful in evaluating the extent of disease above the diaphragm. However, only CT and MRI provide accurate three-dimensional measurements of tumor volume. These modalities may permit identification of intraspinal extension through neural foramina, although myelography may be helpful in some cases. Finally, CT or MRI of the head can further define metastases to the skull, orbits, mandible, or brain.



| Tumor site | Tests |
|---|---|
| Primary | CT or MRI scan[a] with three-dimensional measurements; MIBG scan if available.[b] |
| Metastases | Bilateral posterior iliac crest bone marrow aspirates and trephine (core) bone marrow biopses necessary to exclude marrow involvement. A single positive site documents marrow involvement. Core biopsies must contain at least 1 cm of marrow (excluding cartilage) to be considered adequate.<br>Bone radiographs and either scintigraphy by technetium 99m scan or MIBG scan, if available, are recommended.<br>Abdominal imaging by CT or MRI scan[a] with three-dimensional measurements.<br>Chest radiograph (AP and lateral). Chest CT or MRI scan necessary only if the chest radiograph is positive, or if abdominal mass or lymph node disease extend into chest. |

AP, anteroposterior; CT, computed tomography; MIBG, meta-iodobenzylguanidine; MRI, magnetic resonance imaging.
[a]Ultrasound is considered suboptimal for accurate three-dimensional measurements.
[b]The MIBG scan is applicable to all sites of disease.
From Brodeur GM, Pritchard J, Berthold F, et al. Revisions in the international criteria for neuroblastoma diagnosis, staging and response to treatment. J Clin Oncol 1993;11:1466–1477, with permission.

**TABLE 31-4. MINIMUM RECOMMENDED TESTS FOR DETERMINING EXTENT OF DISEASE**

The potential specificity and sensitivity of MIBG scintigraphy for evaluation of bone and soft tissue involvement by neuroblastoma is attractive. [258,259] MIBG is taken up by catecholaminergic cells, including most neuroblastomas. Thus, radiolabeled MIBG scintigraphy is a specific and potentially sensitive method of assessing the primary tumor and focal metastatic disease. Another modality that shows promise in terms of detection of neuroblastomas is the use of radiolabeled SS analogs.[260,261,262 and 263] Although these analogs provide considerable specificity for neuroblastomas, not all tumors express sufficient receptors to be detectable by this modality. MIBG scintigraphy is readily available in Europe and is currently becoming more widely used in the United States and in some other countries. Those who have experience using MIBG for the evaluation of disease in patients with neuroblastoma find it a very useful technique, so it should be included (when it is available) in the routine evaluation of patients with suspected or proved neuroblastoma. In medical centers in which it is not readily available, technetium 99m diphosphonate scintigraphy remains the standard for evaluation of bone disease, and CT or MRI the standard for evaluation of soft tissue disease.

In the past, there has been considerable variation in the number of bone marrow aspirates or biopsies performed in different institutions for the assessment of bone marrow disease. Indeed, studies suggest that marrow biopsies add substantially to the detection of marrow involvement by tumor compared to marrow aspirates alone.[264,265] Furthermore, it is clear that obtaining more samples will increase the likelihood of detecting marrow involvement, but the clinical relevance of marrow involvement detected with additional studies is uncertain. To provide uniformity, the international conferees on neuroblastoma staging [235,236] recommend two marrow aspirates and two biopsies—that is, one aspirate and one biopsy from each of the iliac crests. Only a single study positive for tumor is required to document bone marrow involvement, but all four studies are required if the findings are negative. It is possible that “neuroblastoma-specific” immunocytology of marrow aspirates will soon obviate the need for marrow biopsies in most patients.[247,248,266]

Other controversial issues include the surgical and histological assessment of lymph node involvement, as well as assessment of the involvement of liver and other organs. Livers with diffuse enlargement or focal lesions should be biopsied, but random biopsies of apparently normal livers probably are unnecessary, especially in older children. Other sites of suspected tumor involvement should be sampled if possible, especially if the findings would alter the stage of the patient. International standardization of surgical approaches to these and other areas should be forthcoming. [267]

## STAGING

In the past, there were two major staging systems used for neuroblastoma throughout the world: (a) the Evans system used by the CCG and others,[203] and (b) the system used by St. Jude Children's Research Hospital and the POG.[268,269] Other systems have been based on these two systems or on the TNM classification.[270,271 and 272] In general, the various staging systems give reproducible results in distinguishing low-stage, good-prognosis patients from high-stage, poor-prognosis patients. Some of the differences between the staging systems are substantial, however, particularly as applied to patients with intermediate stages. For this reason, the results of one group could not be compared readily with another. Therefore, representatives of cooperative groups and countries from around the world met in 1987 and again in 1991 to formulate and then refine the INSS that would lead to uniformity in staging of patients with neuroblastoma for clinical trials and biologic studies around the world (Table 31-5).

| Stage | Description |
|---|---|
| 1 | Localized tumor with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumor microscopically (nodes attached to and removed with the primary tumor may be positive). |
| 2A | Localized tumor with incomplete gross excision; representative ipsilateral nonadherent lymph nodes negative for tumor microscopically. |
| 2B | Localized tumor with or without complete gross excision, with ipsilateral nonadherent lymph nodes positive for tumor. Enlarged contralateral lymph nodes must be negative microscopically. |
| 3 | Unresectable unilateral tumor infiltrating across the midline,* with or without regional lymph node involvement; or lateralized unilateral tumor with contralateral regional lymph node involvement; or midline tumor with bilateral extension by infiltration (unresectable) or by lymph node involvement. |
| 4 | Any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, skin, or other organs (except as defined for stage 4S). |
| 4S | Localized primary tumor (as defined for stages 1, 2A, or 2B), with dissemination limited to skin, liver, or bone marrow† (limited to infants aged <1 yr). |

Note: Multifocal primary tumors (e.g., bilateral adrenal primary tumors) should be staged according to the greatest extent of disease, as defined above, followed by a subscript M (e.g., 3M).
*The midline is defined as the vertebral column. Tumors originating on one side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.
†Marrow involvement in stage 4S should be minimal (that is, less than 10% of total nucleated cells identified as malignant on bone marrow biopsy or on marrow aspirate). More extensive marrow involvement would be considered stage 4. The most sensitive examination can be performed should be negative in the marrow. Cytogenetic studies (see Chapter 62, Section A) on tumor cells may be helpful in establishing the clonal origin of marrow cells. [illegible] Brodeur GM, Pritchard J, Berthold F, et al. Revisions in the international criteria for neuroblastoma diagnosis, staging and response to treatment. J Clin Oncol 1993; 11:1466–1477; with permission.

**TABLE 31-5. INTERNATIONAL NEUROBLASTOMA STAGING SYSTEM**

Stage 1 is a localized tumor that is surgically resected.[235,236] Microscopic residual tumor at the margins is allowed. Stage 2 represents localized tumors with gross residual disease (2A) or localized tumors with ipsilateral lymph node involvement, regardless of resectability (2B). At the present time, it does not appear that there is a substantial prognostic distinction between 2A and 2B, at least in infants.[273] Stage 3 tumors show invasion across the midline, either on the basis of direct extension or by lymph node involvement. Most stage 3 tumors arise in the abdomen, because tumors crossing the midline by contiguous infiltration or by lymph node involvement are less common in the thorax. Infiltration is meant to indicate contiguous invasion of tumor across the midline (defined as the vertebral column) rather

than a pedunculated tumor that hangs over the midline.

Patients with disseminated disease involving distant lymph nodes, bone, bone marrow, liver, or other organs are categorized as having stage 4 disease (except as defined in stage 4S). This definition of stage 4 is essentially identical to stages IV and D, respectively, by the two major staging systems used previously. [203,239] There is some evidence that patients who have stage 4 on the basis of distant lymph node, liver, or marrow involvement (excluding 4S), especially patients younger than 2 years old, do better than those who have stage 4 disease on the basis of cortical bone involvement. [271,272] Because these distinctions may impact prognosis or choice of therapy, the criteria by which patients are considered to have stage 4 should be recorded. However, such distinctions may also be affected or obliterated by improvements in treatment.

Stage 4S (equivalent to IV-S or DS) has been retained as a distinct stage, based on the favorable outcome generally experienced with these patients, [274,275,276 and 277] and because of recent biologic evidence distinguishing these patients from infants with conventional stage 4 disease. For example, the majority of stage 4S tumors has a hyperdiploid DI, and less than 10% have *MYCN* amplification, in contrast to tumors from stage 4 infants, in which the DI is more often diploid and *MYCN* is amplified in approximately one-third. [97] The issue of bone marrow involvement has been clarified to mean that less than 10% of nucleated marrow cells are malignant cells. [236] Patients with more extensive marrow involvement should be classified as stage 4.

The efficacy of these staging definitions to define prognostic subsets of patients has been recently evaluated by POG and CCG by applying the INSS criteria to prospectively collected surgicopathologic data. In the POG study, 596 children with neuroblastoma were analyzed. [273] Patients with tumors that were localized or were disseminated to distant sites composed the most and least favorable groups, respectively. There was no difference in the EFS for infants having tumors with INSS stages 2A, 2B, or 3. In contrast, older children with INSS stage 2A/2B disease had a significantly better EFS when compared to those with INSS stage 3 tumors ( $p$ <.019). Of the 424 cases reviewed in the CCG study, concordance between INSS stages 1 and 4 and Evans stages I and IV was noted. [278] Furthermore, of the Evans stages II (n = 144) and III (n = 193) tumors, 89 were assigned to INSS stage 1 (relapse-free survival 82% to 85%) and 112 to INSS stages 2A/2B (relapse-free survival 61% to 70%). This experience with the INSS suggests that these new criteria clearly provide prognostic information that is at least equivalent or superior to the staging systems used previously. This is an important step toward furnishing a stable clinical background on which multivariate analyses can be performed to identify biologically based risk groups.

## DEFINITIONS OF RESPONSE TO TREATMENT

A variety of terms have been used to report the response of neuroblastoma patients to a given treatment regimen (see definitions and Table 31-6). [235,236] Despite their general use, the same term may have a different meaning when used by different groups. This is due in part to differences in the number and type of tests used for evaluation and the time at which the response is evaluated, which often varies considerably from one institution to another. The same tests that are used for determining extent of disease (Table 31-4) should be used to assess response of primary and metastatic sites to treatment. Table 31-6 lists internationally proposed criteria to determine response to therapy in patients with neuroblastoma. [236] It is important to note that a given level of overall response involves thorough assessment of both primary and metastatic sites. For example, a CR overall requires that the primary tumor and all metastatic sites fulfill CR criteria. A CR in metastatic sites and a PR in the primary tumor would be considered a PR overall. These criteria are very similar to the recently proposed response evaluation criteria in solid tumors that are being recommended for future clinical trials of pediatric cancers. [279] Evaluations for response in newly diagnosed patients are recommended at the end of induction (usually 3 to 4 months), at the end of treatment (usually 8 to 12 months), before and after surgical procedures, before stem cell transplantation, and as indicated clinically. Three-dimensional measurements should be possible for primary tumors and many metastatic lesions based on CT scans or other diagnostic imaging modalities.

| Response | Primary[a] | Metastases[a] | Markers[a] |
|---|---|---|---|
| Complete response | No tumor | No tumor (e.g., chest, abdomen, liver, bone, bone marrow, nodes) | MIBI/MMA normal |
| Very good partial response | Reduction >90% but <100% | No tumor (as above except bone); no new bone lesions; all preexisting lesions improved | MIBI/MMA decreased >90% |
| Partial response | Reduction 50-90% | No new lesions; 50-90% reduction in measurable sites; 0-ii bone marrow samples with tumor; bone lesions same as very good partial remission. | MIBI/MMA decreased 50-90% |
| Mixed response | — | No new lesions; >50% reduction of any measurable lesion (primary or metastases) with <50% reduction in any other; <25% increase in any existing lesion.[b] | — |
| No response | — | No new lesions; <50% reduction but <25% increase in any existing lesion.[b] | — |
| Progressive disease | — | Any new lesion; increase of any measurable lesion by >25%; previous negative marrow positive for tumor. | — |

[a]Evaluations of primary and metastatic disease as outlined in Table 31-5.
[b]Quantitative assessment does not apply to marrow disease.
From Brodeur GM, Seeger RC, Barrett A, et al: International criteria for diagnosis, staging, and response to treatment in patients with neuroblastoma. J Clin Oncol 1988;6:1874-1881; and Brodeur GM, Pritchard J, Berthold F, et al: Revisions in the international criteria for neuroblastoma diagnosis, staging and response to treatment. J Clin Oncol 1993;11:1466-1477, with permission.

**TABLE 31-6. PROPOSED DEFINITIONS OF RESPONSE TO TREATMENT**

## PROGNOSTIC CONSIDERATIONS

There has been substantial progress made in risk stratification for neuroblastoma patients based on the analysis of a panel of clinical and biologic variables. Indeed, there is an evolving list of prognostic markers that should be analyzed at diagnosis for each newly diagnosed patient to best assign intensity of therapy. This list will be refined over time based on prospective evaluation of additional variables postulated to be of clinical utility.

### Clinical Variables

The most important clinical variables predictive of disease outcome are the age of the patient and stage of disease at diagnosis. [191,231,280,281,282,283,284,285 and 286] These variables are of proven prognostic utility and will be the cornerstone of any risk stratification schema. For example, the 4-year EFS probability for patients with INSS stage 3 neuroblastoma ranged from 98% for infants to 75% for patients older than 2 years at diagnosis in a recent CCG study. [286] Tumor site has also been proposed to be prognostically important, with adrenal tumors reported to be associated with a more aggressive clinical course. In addition, for the subset of patients who present with metastatic disease, cortical bone involvement appears to be more closely associated with *MYCN* amplification and a poorer survival probability. [231] However, neither primary nor metastatic disease site appears to have an independent influence on outcome.

### Biologic Variables

Several previously studied biologic variables (pathology, serum markers, genetic features) appear to have prognostic value in patients with neuroblastoma. A new histopathologic classification, the International Neuroblastoma Pathology Classification, based on the original Shimada system, is likely to become the new international standard. The serum markers include ferritin, NSE, a cell membrane ganglioside ($G_{D2}$), and LDH. The genetic features of the tumor that have been proposed as prognostic markers include tumor cell DI, *MYCN* gene copy number, deletion or LOH involving 1p, unbalanced gain of 17q, and relative expression of the neurotrophin receptor *TRKA*. No study to date has examined all variables in a large set of patients, so it is difficult to say which single variable or combination of variables are the most powerful predictors of outcome in addition to the more conventional clinical features of patient age and stage.

#### *Tumor Pathology*

Differentiated histology, such as in ganglioneuroblastoma, generally is associated with localized tumors, but this type of histologic classification does not have prognostic value that adds significantly to age and stage. More detailed analysis of histology by the classifications of Shimada and colleagues [189] or Joshi and colleagues [190] have provided important prognostic information (see Pathology). However, these are being replaced by a new International Pathology Classification System that should provide worldwide uniformity in the application of this variable ( Table 31-7). [195,196]

| International Neuroblastoma Pathology Classification | | Original Shimada classification | Prognostic group |
|---|---|---|---|
| Neuroblastoma | Schwannian stroma-poor[1] | Stroma-poor | |
| Favorable | | Favorable | Favorable |
| <1.5 yr | Poorly differentiated or differentiating and low or intermediate MKI tumor | | |
| 1.5–5.0 yr | Differentiating and low MKI tumor | | |
| Unfavorable | | Unfavorable | Unfavorable |
| <1.5 yr | Undifferentiated tumor[2] High MKI tumor | | |
| 1.5–5.0 yr | Undifferentiated or poorly differentiated tumor Intermediate or high MKI tumor | | |
| >5 yr | All tumors | | |
| Ganglioneuroblastoma, nodular | (Composite Schwannian stroma-rich/stroma-dominant with stroma poor) | Stroma-rich nodular (unfavorable) | Unfavorable |
| Ganglioneuroblastoma, intermixed | Schwannian stroma-rich | Stroma-rich intermixed (favorable) | Favorable |
| Ganglioneuroma | Schwannian stroma-dominant | | |
| Maturing | | Well differentiated (favorable) | Favorable[3] |
| Mature | | Ganglioneuroma | |

MKI, mitotic karyorrhexis index.
[1]Subtypes of neuroblastoma are described in detail elsewhere.[16]
[2]Rare subtype, especially diagnosed in this age group. Further investigation and analysis required.
[3]Prognostic grouping for these tumors' prognosis is unrelated to patients' age.
Modified from Shimada H, Ambros IM, Dehner LP, et al. The International Neuroblastoma Pathology Classification (the 'Shimada system'). Cancer 1999;86:364–372, with permission.

**TABLE 31-7. INTERNATIONAL NEUROBLASTOMA PATHOLOGY CLASSIFICATION**

### *Serum Markers*

Serum markers that have been used in the past to predict outcome or follow disease activity include ferritin, NSE, LDH, and $G_{D2}$. Although each of these markers has prognostic value, at least in subsets of patients, none appears to have overall prognostic value comparable to newer genetic markers (e.g., *MYCN* amplification), and none is being used currently by major groups for risk stratification.

#### Ferritin

Ferritin may be increased in some patients with actively growing neuroblastomas, and those with increased levels have a much worse prognosis. [287,288,289 and 290] Serum ferritin may be particularly useful as a prognostic marker when combined with tumor histology. [192]

#### Neuron-Specific Enolase

NSE is a cytoplasmic protein with enolase activity that is associated with (but not restricted to) neural cells. Analysis of serum NSE from patients with neuroblastoma has indicated that survival is substantially worse in advanced patients with increased serum NSE levels. [291,292,293 and 294]

#### Lactate Dehydrogenase

Although not specific to neuroblastoma, serum LDH level has been used as a prognostic marker for neuroblastoma. Increased LDH levels may be a reflection of rapid cellular turnover or of large tumor burden. Indeed, assessment of serum LDH added significant predictive value to the clinical features of age and stage in some studies.[295,296,297 and 298]

#### $G_{D2}$

$G_{D2}$ is a disialoganglioside on most neuroblastoma cells to which several independently derived monoclonal antibodies have been raised. The presence of this ganglioside is useful for identifying neuroblastoma cells, and increased levels have been found in the plasma of patients with neuroblastoma.[299,300,301,302,303,304,305,306,307,308,309,310,311 and 312] Indeed, gangliosides shed by tumor cells may play a role in accelerating tumor progression. [303,304] Antibodies against $G_{D2}$ may be useful for therapy of neuroblastoma as well (see below).

### *Genetic Markers*

A variety of genetic markers have been identified that are highly correlated with tumor subsets that have characteristic clinical behavior. Those that have been shown to have prognostic value in several groups or countries include *MYCN* amplification, tumor cell DI, and allelic loss of 1p (see below). Others that are still under investigation or validation include unbalanced gain of 17q, allelic loss of 11q, and other patterns of genetic change.

#### *MYCN* Amplification

*MYCN* amplification in tumor cells is found predominantly in patients with advanced stages of disease and is generally associated with rapid tumor progression and a poor prognosis.[97,98,101,102 and 103,107,108 and 109,286,305,306 and 307] This adverse prognostic affect is especially evident in infants with stage 4 disease (see Fig. 31-12). Although not all patients with a poor outcome have *MYCN* amplification, the majority of patients with amplification treated with contemporary therapies has rapid progression and die, even patients with stage 4S disease.[98,308,309] There have been notable exceptions with localized disease [310,311] or stage 4S,[312] which may indicate that other favorable biologic factors override the negative impact of *MYCN* amplification on outcome. Nonetheless, *MYCN* amplification is considered a paradigm of the utility of tumor-specific genetic alterations for prognosis and is used currently used by all international cooperative groups for treatment stratification (see below).

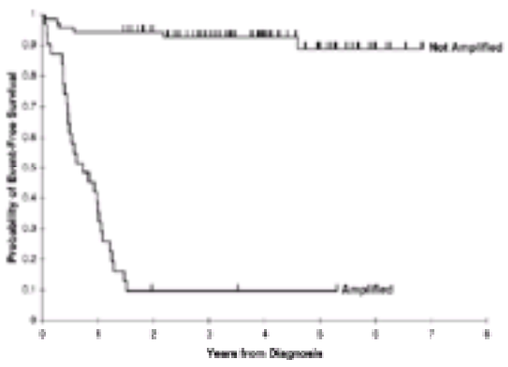



**FIGURE 31-12.** EFS for 102 infants with stage 4 neuroblastoma, stratified by *MYCN* gene status.[303] Not amplified: n = 71 pts; amplified: n = 31 pts; *p* <.0001. (Figure redrawn with permission from Mary Lou Schmidt, University of Illinois, Chicago.)

#### Hyperdiploid Karyotype

Tumor cells with a hyperdiploid karyotype (or increased DI) [96,97,104,105 and 106,160,305,313,314] are associated with a favorable outcome in infants with neuroblastoma. Look and colleagues[96,97] originally showed that infants with neuroblastoma and a tumor DI greater than 1 had a better outcome compared to infants with tumor DI of 1. Bowman and colleagues[315] reported the results of a prospective study of 113 infants with INSS stages 2A, 2B, 3, 4, and 4S in which treatment was determined according to tumor ploidy. After five cycles of cyclophosphamide/doxorubicin, CRs were attained in 98% of the 85 infants with tumor DI greater than 1, and all are surviving free of disease with a follow-up of 11 to 30 months. In contrast, only one-third of the 28 infants with a tumor DI of 1 achieved CR after treatment with cisplatin/teniposide, and 25% survived free of disease. In a study of 110 infants with stage 4S neuroblastoma, a DI of 1 was significantly associated with a decreased OS probability in univariate analysis, but four of five patients with a diploid DI also had *MYCN* amplification.[98] Prospective analysis of this prognostic variable is ongoing within the new COG, which represents a fusion of POG and CCG with the National Wilms' Tumor Study Group and the Intergroup Rhabdomyosarcoma Study

Group.

### Chromosomal Deletion of Allelic Loss

Chromosomal deletion or allelic loss has been shown in several studies to be correlated with a poor survival. [141,146,148,150,156,316] There is a strong correlation between 1p LOH and high-risk features, such as age older than 1 year at diagnosis, metastatic disease, and unfavorable histology. [150,152,153] In addition, there is a strong association between *MYCN* amplification and 1p deletion. Some cases of 1p deletion do not have *MYCN* amplification, but virtually every case with *MYCN* amplification has 1p deletion.[148,149 and 150,156,157 and 158,163] It remains controversial whether 1p allelic loss has independent prognostic significance after correction for the prognostic impact of *MYCN* amplification as well as the other currently used prognostic variables, [141,152,153,316,317] although recent data suggest that this abnormality might be an independent predictor for disease relapse in patients with locoregional disease. [150,318]

Although there was no survival disadvantage for patients whose tumors had 11q LOH in univariate analyses, there was a significant decrease in OS probability when the subset of patients without *MYCN* amplification was analyzed.[162,319] Thus, 11q is one of the most common regions of allelic deletion currently identified in primary neuroblastomas, and it may be a marker of an unfavorable phenotype independent of *MYCN* amplification. Allelic loss of 14q also appears to be inversely correlated with *MYCN* amplification but most likely has limited prognostic significance utility. [166]

#### *Other Biologic Markers*

Other biologic factors related to gene expression have been proposed to provide additional prognostic information for neuroblastoma patients. These include expression of the NGF receptor *TRKA*,[70,71,72,73 and 74] expression of genes related to the multidrug resistance phenotype (*MDR1* and *MRP*),[320,321,322,323,324 and 325] genes related to invasion and metastasis (nm23 and CD44),[326,327,328 and 329] or other genes related to neural differentiation (*HRAS*, *PTN*).[64,126,330] The data for *TRKA* are quite strong and consistent among the five groups that have now studied this feature. [70,71,72,73 and 74] The preliminary results investigating the prognostic value of *MRP* expression are also quite promising,[325] but there are conflicting results regarding the prognostic value of *MDR1* expression. The other biologic markers have shown promise, but most have been studied in only a limited number of cases or institutions.

## PRINCIPLES OF INITIAL THERAPY

The treatment modalities traditionally used in the management of neuroblastoma are surgery, chemotherapy, and radiotherapy. The role of each is determined by the anticipated clinical behavior of the tumor in individual cases considering stage, age, and biologic features.

### Surgery

Surgery plays a pivotal role in the management of neuroblastoma, both for diagnosis and for treatment. [331] The goals of primary surgical procedures, performed before any therapy, are to establish the diagnosis, to provide tissue for biologic studies, to stage the tumor surgically, and to attempt to excise the tumor without injury to vital structures. In delayed primary or second-look surgery, the surgeon determines response to therapy and removes residual disease when possible.

The operative protocol for surgicopathologic staging as recommended by the INSS criteria (see the section on staging) should include the following:

- The resectability of primary or metastatic tumor should be determined by tumor location, mobility, relationship to major vessels and nerves, ability to control blood supply, presence of distant metastases, and overall prognosis of the patient. With the efficacy of modern chemotherapy to consolidate and reduce the size of large primary tumors and lymph node metastases, sacrifice of vital structures to achieve resection at diagnosis should be avoided. This is particularly so in young children in whom the prognosis is excellent in most cases.
- Nonadherent, intracavitary lymph nodes should be sampled. Gross examination during surgery may be inaccurate for detecting or ruling out lymph node metastases in up to 25% of cases.[332] The status of lymph nodes adherent to and removed en bloc with the primary tumor seem to have little relevance in predicting outcome of the patient.[332] Ideally, lymph nodes superior and inferior to the primary tumor should be sought and sampled, with documentation of location. Problematic situations for lymph node sampling include patients with high thoracic, low cervical, or large abdominal primary tumors that are unresectable. In these cases, access to lymph nodes may be difficult. Because there are other prognostic factors that may be assessed in these cases, the value of additional information regarding lymph node involvement is questionable.
- In cases of abdominal tumors without metastatic disease by clinical evaluation, biopsy of the liver has been advocated at initial surgery. The usefulness of *random* liver biopsy was questioned in a study of 54 children older than 1 year at diagnosis with POG stage C disease (INSS stages 2B and 3) arising in the abdomen.[333] This study concluded that liver biopsy was only indicated in older children if there was gross or radiologic evidence of metastasis. In contrast, diffuse involvement of the liver may be missed by radiologic studies in infants, so random liver biopsies are still indicated in these patients. [331]

The importance of gross total resection in the management of disseminated neuroblastoma remains controversial. Haase and coworkers [334] noted improved disease-free survival in 39 children with complete resection versus 23 patients undergoing partial resection. Neither the timing of the surgery nor tumor *MYCN* copy number predicted resectability of primary tumors. In a retrospective review of 70 cases at Memorial Sloan-Kettering Cancer Center, gross total resection of the primary tumor correlated with improved survival ($p = .03$).[285] Due to the changing intensity of chemotherapy given over the time period of the review, however, it was not possible to detect whether surgical resection was an independent prognostic factor. Chamberlain and colleagues [335] noted superior rates of survival at 3 years (40% vs. 15%) in patients undergoing complete versus partial surgical resection. In contrast, the experience at Great Ormond Street did not confirm that such radical surgery provides a survival advantage in these patients. [336,337] Finally, Nakagawara and colleagues[338] noted that the degree of surgical resection of the primary tumor was directly related to improved survival in patients with Evans stages III and IV, *MYCN* amplified tumors, but not in tumors with normal *MYCN* copy number. Further examination of this area is needed, but it is likely that the biology of tumors may affect their resectability, accounting for the differences seen in some studies.

Surgical complications in neuroblastoma have been reported at rates from 5% to 25%. [269,333,339,340] The incidence is highest with aggressive attempts to resect abdominal tumors at diagnosis. Commonly encountered problems include nephrectomy, operative hemorrhage, postoperative intussusception or adhesions, injury to renal vessels with subsequent renal failure, and neurologic deficits such as Horner's syndrome. Because complications are more frequent in infants, who have a substantially better survival, avoidance of surgical risk is particularly important. Complications generally are lower for delayed or second-look procedures, after tumor shrinkage by chemotherapy.[339]

### Radiation Therapy

Neuroblastoma is generally considered a radiosensitive tumor, although local control of neuroblastoma on a clinical level has been variable. [341,342] Accepted tumoricidal doses of ionizing radiation range from 15 to 30 Gy, depending on patient age, tumor volume, and tumor location. [343,344] Fractionation of doses ranges from 150 to 400 cGy, again depending on tumor volume. Historically, radiation has been used in the multimodality management of residual neuroblastoma, bulky unresectable tumors, and disseminated disease. More recently, the role of radiotherapy in neuroblastoma continues to be refined with the improvement in multi-agent chemotherapy and the increasing trend toward developing risk-related treatment groups based on age, stage, and biologic features.

The role of radiation therapy for patients with locoregional disease has evolved. From a randomized trial in children with regional lymph node metastases (INSS stages 2B, 3), Castleberry and colleagues[333] initially reported statistically superior rates of CR (76% versus 46%, $p = .013$), EFS (59% versus 32%, $p = .009$) and survival (73% versus 41%, $p = .008$) in patients receiving low-dose, sequential cyclophosphamide/doxorubicin [345] in combination with local radiation (24 to 30 Gy) compared to the same chemotherapy alone. However, in the context of more dose-intensive chemotherapy, and accounting for the status of *MYCN* copy number, this may no longer be true. Strother and coworkers[346] reported a subsequent study from POG in which INSS stage 2B/3 tumors were treated with high-dose cisplatin and etoposide alternated with low-dose sequential cyclophosphamide plus doxorubicin. Radiation was given only if a CR was not achieved after 15 weeks of treatment plus second-look surgery. Sixteen of 21 patients without *MYCN* amplified tumors remain free of disease, most without radiotherapy, compared to only 1 of 11 with tumors having *MYCN* amplification. A similar adverse effect of *MYCN* amplification on outcome in stage 2 tumors was observed by West and colleagues [284] at the Dana-Farber Cancer Institute.

A clear indication for radiation therapy is for neonates with INSS stage 4S neuroblastoma who develop respiratory distress secondary to hepatomegaly and for whom

treatment with chemotherapy is ineffective.[276,277,282,295,347] Effective doses are 3 to 6 Gy in single or multiple fractions.[341] However, considering the potential long-term side effects, chemotherapy alone should remain the initial approach in these patients.

Total body irradiation represents another potential use of radiation in neuroblastoma patients. Doses of 7.5 to 12.0 Gy given in three to five fractions have been used as part of many preparative regimens for ABMT or stem cell transplantation.[348,349 and 350] The benefits of high-dose chemoradiotherapy relative to marrow ablative chemotherapy alone in the ABMT setting have not been established.

Traditionally, radiation alone or in combination with laminectomy has been used to rapidly reduce cord compression in children with dumbbell extension of neuroblastoma associated with spinal symptoms. Radiation doses used range from 7.5 to 30.0 Gy.[351] Although effective, both modalities are associated with a significant incidence of vertebral body damage, including growth arrest and instability leading to scoliosis. In newly diagnosed patients, the use of chemotherapy alone has been reported to be an efficacious alternative associated with fewer long-term side effects.[352,353,354 and 355]

Finally, for treating problematic metastatic disease at diagnosis or for palliative management of pain in end-stage disease, radiation in daily fractions to total doses of 4 to 32 Gy usually affords immediate relief of symptoms and may result in prolonged control at the site treated.[342]

**Chemotherapy**

Chemotherapy is the predominant modality of management in neuroblastoma patients who have intermediate- or high-risk disease (discussed in more detail in the section on risk-related therapy). Single-agent, phase II trials conducted in patients with recurrent or advanced neuroblastoma have identified a number of effective drugs that form the backbone of current induction chemotherapy regimens.[356,357,358,359,360,361,362,363,364,365,366,367,368,369,370,371,372,373,374,375,376,377,378,379,380 and 381] In particular, these data have established the activity of alkylating agents and platinum analogs in this disease. Cyclophosphamide, cisplatin, doxorubicin, and the epipodophyllotoxins have yielded CR and PR rates ranging from 34% to 45% in patients with refractory disease, and they have become the cornerstone of most multi-agent regimens.

The concept of an initial phase II window to evaluate single agents in previously untreated high-risk cancer patients has been applied to children with disseminated neuroblastoma.[381,382,383 and 384] Using this study design, it has been possible to determine response rates and acute toxicity of several agents in the context of chemotherapeutically naive tumors and patients who are not chronically ill from tumor or previous treatment ( Table 31-8). One concern regarding such a design is that single-agent treatment may have an adverse effect on subsequent multi-agent therapy. In the European study reported by Kellie and colleagues,[382] only 12 of 18 children had a response to OPEC or similar therapy after an initial two courses of ifosfamide alone, which appeared to be inferior to previous experience. However, in the POG experience, in which patients received similar multidrug induction chemotherapy with or without upfront single-agent treatment, there was no evidence of worsening response rates or outcome due to the use of the phase II window.[383,384] Such a study design seems an appropriate adjunct to future clinical trials for high-risk disease.

| Study | Reference | Drug and schedule | No. patients | CR + PR (%) |
|---|---|---|---|---|
| POG 8741 | 383 | Carboplatin 560 mg/m² | 49 | 54 |
| | | Ifosfamide 2 g/m² per d × 4 | 52 | 45 |
| | | Cisplatin 100 mg/m² | 54 | 40 |
| | | Epirubicin 90 mg/m² | 23 | 14 |
| European Neuroblastoma Study Group 3A | 382 | Ifosfamide 3 g/m² per d × 2 | 18 | 44 |
| POG 9341 | 384 | Topotecan 2 mg/m² per d × 5 | 33 | 38 |
| | | Topotecan 2 mg/m² per d × 5 and cyclophosphamide 250 mg/m² per d × 5 | 38 | 29 |
| | | Taxol 200 mg/m² | 33 | 18 |

CR, complete response; PR, partial response.

**TABLE 31-8. ACTIVITY OF AGENTS STUDIED IN PHASE II WINDOWS FOR NEWLY DIAGNOSED HIGH-RISK NEUROBLASTOMA PATIENTS**

**Concept of Risk-Related Therapy**

A large body of data supports the hypothesis that the clinical behavior of human neuroblastoma may be reliably predicted based on the analysis of a panel of prognostic variables.[102,319] Thus, most pediatric oncology clinical trials groups currently stratify patients into risk groups based on analysis of well-defined prognostic factors. The COG Risk Stratification System is based on the experience of both the POG and CCG ( Table 31-9).[269,286,306,311,333,385,386] This system uses analysis of the clinical factors of patient age at diagnosis and INSS stage and the biologic factors of tumor histopathology, DI, and *MYCN* amplification status to assign patients to one of three distinct risk groups (low, intermediate, and high). A major goal of ongoing clinical trials is to prospectively evaluate “candidate” prognostic markers, such as 1p36 LOH, unbalanced 17q gain, and *TRKA* expression for independent influence on patient outcome.

| Stage[a] | Low risk | Intermediate risk | High risk |
|---|---|---|---|
| 1 | All | None | None |
| 2A, 2B | Age < 1 yr; or<br>Age 1–21 yr; *MYCN* non-*AMP*; or<br>Age 1–21 yr; *MYCN AMP* and FH[c] | None | Age 1–21 yr with *MYCN AMP* and UH |
| 3 | None | Age < 1 yr and *MYCN* non-*AMP*; or<br>Age 1–21 yr; *MYCN* non-*AMP* and FH | Age 0–21 yr with *MYCN AMP*; or<br>Age 1–21 yr; *MYCN* non-*AMP* and UH |
| 4 | None | Age < 1 yr with *MYCN* non-*AMP* | Age < 1 yr and *MYCN AMP*; or age 1–21 yr |
| 4S | *MYCN* non-*AMP*; FH; DI < 1 | *MYCN* non-*AMP*; UH or DI > 1 | *MYCN AMP* |

[a]International Neuroblastoma Staging System.[26]
[b]*MYCN* amplification status: *AMP*, amplified; non-*AMP*, not amplified.
[c]Histopathologic classification[98]: FH, favorable histology; UH, unfavorable histology.

**TABLE 31-9. PROPOSED NEUROBLASTOMA RISK GROUPS BASED ON CLINICAL AND BIOLOGIC TUMOR FEATURES**

***Treatment of Low-Risk Disease***

Included in the low-risk disease group are the following patients: all patients with INSS stage 1 disease; patients with INSS stage 2 disease, except those patients older than 1 year at diagnosis with tumor *MYCN* amplification *and* unfavorable Shimada pathology; and infants with 4S disease with tumors with hyperdiploidy, favorable Shimada, and single-copy *MYCN* (Table 31-9).

Treatment of low-risk neuroblastoma consists of surgical removal of the primary tumor. Unique to neuroblastoma, a complete resection is unnecessary in the setting of localized disease and favorable biologic features. Patients with INSS stage 1 tumors (gross total resection) can be expected to have a relapse-free survival probability of greater than 90%, regardless of age.[311,387,388 and 389] Perez and colleagues[311] recently reported a 93% EFS rate for 141 Evans stage I patients. Six of the ten disease recurrences occurred at distant sites, but two of these occurred in patients with *MYCN* amplified tumors (see below). The OS rate for this cohort of patients was 99%. These data support surgery alone as effective initial therapy of INSS stage 1 neuroblastomas. Local recurrences can be managed with second surgeries, but even metastatic recurrences are often salvageable with chemotherapy.

Surgery alone is also the initial treatment of choice for the majority of patients with INSS stage 2 neuroblastoma. Historically, many of these patients were treated with chemotherapy, and survival results were excellent.[390] Several single institutions and cooperative groups have reported results of treatment with surgery only, however, and survival does not appear to be compromised.[311,387,389,391,392] In a recent prospective CCG study, 233 Evans stage II patients (56% INSS stage 2) with single-copy *MYCN* were managed with surgery alone.[311] Although the 4-year EFS rate was 81%, the majority of patients who experienced disease relapse was salvaged, as the 4-year OS rate was 98%. Thus, even in the setting of macroscopic residual disease, adjuvant chemotherapy or radiotherapy does not seem warranted for the vast majority of INSS stage 2 patients.

It remains controversial how best to manage the rare patients with INSS stage 1 or 2 neuroblastomas and *MYCN* amplification. Cohn and colleagues[393] reported that five of six patients with *MYCN* amplified INSS stage 1 (n = 3) or stage 2A (n = 3) tumors survived without evidence of disease at 7+ to 38+ months. However, Perez and colleagues[308] recently showed that four of seven INSS stage 1 (n = 4, all infants) and stage 2b (n = 3, two infants) cases with *MYCN* amplification had metastatic relapse at 2 and 22 months from surgery, and three of these patients died from disease progression. Likewise a French cooperative group reported that three of four patients with INSS stage 1 or 2 neuroblastoma and *MYCN* amplification relapsed and died from disease progression.[391] Thus, this rare clinical situation needs prospective evaluation under uniform therapeutic recommendations to arrive at a consensus treatment strategy.

The majority of patients with INSS stage 4S disease falls into the low-risk category. Retrospective analyses have shown OS rates ranging from 57% to 97% for stage 4S patients taken as a whole.[275,276] and [277,347,392,394,395,396,397,398,399] and [400] Recent prospective analyses have confirmed these observations and show OS probabilities of 85% (n = 110) to 92% (n = 80).[98,401] However, it has become clear that some infants with this unique pattern of disease are at much higher risk for life-threatening complications. First, the subset of patients diagnosed in the first 2 months of life appears particularly vulnerable to respiratory compromise secondary to rapidly progressive hepatomegaly.[98,400,401] In addition, there is a subset of stage 4S patients with unfavorable biologic features such as *MYCN* amplification that often shows rapid tumor progression or eventual disease relapse similar to “true” stage 4 disease.[98,308,401] These data emphasize the importance of biologic assessment of tumor tissue in patients with stage 4S disease.

The indications for intervening and the methods of managing infants with stage 4S neuroblastoma are becoming standardized. A diagnostic biopsy is indicated for evaluation of tumor biologic features, but resection of the primary tumor does not appear to influence outcome.[98,401] Thus, the safest surgical procedure, such as removal of a subcutaneous nodule, should be performed. Patients with favorable biologic features should be observed closely for symptomatology from tumor expansion, especially when younger than 2 months at diagnosis. If respiratory compromise becomes evident, moderately intensive chemotherapy is indicated, such as that currently recommended for intermediate-risk disease.[98,282,295,347,401] Radiation therapy should be reserved for those patients who progress despite chemotherapy. If necessary, a minimal dose of radiation, such as 450 to 600 cGy in three to four fractions is generally sufficient to halt tumor progression and induce regression. Lastly, the rare stage 4S patients with unfavorable biologic features should be considered for either intermediate-risk (diploidy or unfavorable Shimada pathology) or high-risk (*MYCN* amplification) treatment strategies.

Although neuroblastoma patients with intermediate- or high-risk disease features may present with intraspinal extension and cord compression, this clinical situation is most frequently identified in children who otherwise would be considered low risk. Many of these patients are quite young, so extensive laminectomies or radiation to the spine can result in long-term morbidity. Several groups have reported their retrospective experience with the management of intraspinal neuroblastoma.[352,353,354] and [355,402] In general, these studies show no survival or neurologic outcome advantage for neurosurgical decompression or external beam radiation therapy. In addition, Hoover and colleagues[355] documented mild to severe spinal deformities in 11 of 15 patients treated initially with laminectomy. The French NBL90 study prospectively evaluated the role of initial chemotherapy for 42 nonmetastatic neuroblastomas presenting with intraspinal extension.[354] They showed that chemotherapy alone was successful in reducing the tumor volume in 58% of cases and was associated with improved neurologic function in 92%. Thus, chemotherapy appears to be a safe and effective initial modality to manage spinal canal invasion, and it has less long-term morbidity than do surgery or radiation therapy.

### *Treatment of Intermediate-Risk Disease*

Included in the intermediate-risk disease group are the following patients: INSS stage 3 patients younger than 1 year with nonamplified *MYCN*, INSS stage 3 patients older than 1 year with nonamplified *MYCN* and favorable Shimada pathology, stage 4 infant patients with nonamplified *MYCN,* and nonamplified stage 4S patients with unfavorable Shimada histopathology, a DI of 1, or both (Table 31-9). The group of patients currently defined as INSS stage 3 have been treated in a heterogeneous fashion in past studies due to differences in the staging systems used by the various cooperative groups. Therefore, treatment results have been difficult to compare. International acceptance of the INSS system should allow for a more uniform approach to treatment planning for this subset of patients.

Matthay and colleagues[286] recently reported the CCG experience with Evans stage III disease, and because 92% of their patients were INSS stage 3, results can be extrapolated to the current risk stratification system. Of 228 Evans stage III patients prospectively evaluated for risk status by analysis of age, stage, *MYCN* amplification status, Shimada pathology,[403] and serum ferritin,[288] 143 met the current criteria for intermediate-risk disease. These 143 patients were treated with moderately dose-intensive chemotherapy including cyclophosphamide, doxorubicin, cisplatin and etoposide, as well as local radiation for any gross residual disease following delayed surgery. Patients with Evans stage III disease and normal *MYCN*, favorable Shimada, and low serum ferritin had a 4-year EFS of 100%. Infants with Evans stage III disease and at least one unfavorable biologic feature had 4-year EFS and OS rates of 90% and 93%, respectively. In contrast, patients with Evans stage III disease older than 1 year at diagnosis with at least one unfavorable biologic feature had 4-year EFS and OS rates of 65% and 75%, respectively, despite a much more dose-intensive treatment regimen. In multivariate analysis, age at diagnosis and *MYCN* status were the only factors independently prognostic in this group of patients.

The role of radiotherapy for advanced locoregional neuroblastoma remains controversial. Castleberry and colleagues[333] previously showed that addition of 24 to 30 Gy of external beam radiotherapy to the primary tumor bed and regional lymph nodes following moderately aggressive chemotherapy improved overall rates of both local and metastatic relapse for children with POG stage C disease (INSS stages 2B and 3). In this randomized study, 46% of 28 patients treated with chemotherapy alone achieved CR and 32% were free of disease at 2 years. In comparison, 75% of 29 children treated with chemotherapy plus radiation therapy achieved a CR, and 59% were free of disease at 2 years. In contrast, de Bernardi and colleagues[270] found no advantage for radiotherapy in a randomized trial (chemotherapy with or without radiotherapy) in 29 nonmetastatic patients with residual tumor or positive lymph nodes (INSS stages 2 and 3) following initial surgery. Furthermore, nonrandomized addition of radiation therapy for patients with gross residual disease following second surgery for Evans stage III disease had no apparent influence on disease outcome.[286] Currently, most cooperative groups are withholding radiation therapy for the majority of intermediate-risk patients, except for patients with disease progression despite surgery and chemotherapy or patients with unresectable primary tumors with unfavorable biologic features at the end of chemotherapy.

The majority of infants with INSS stage 4 neuroblastoma is currently categorized as intermediate risk. It is now clear that infants with metastatic neuroblastoma that is *MYCN* amplified uniformly have a highly malignant clinical course,[306,307,386,404] and therefore should be categorized as high risk. However, infants with neuroblastomas that do not have amplified *MYCN* typically have a much less aggressive clinical course and respond to moderate intensity chemotherapy. Schmidt and colleagues[303] reviewed the recent CCG experience and reported a 3-year EFS rate of 93% for infants with *MYCN* single-copy neuroblastomas treated with moderately intensive chemotherapy. This contrasts with the 10% EFS noted for those infants with *MYCN*-amplified tumors, many of whom were treated with much more intensive therapy (Fig. 31-12). These data compare favorably to previous reports from the POG,[398,399] perhaps because the CCG study used a somewhat more intensive chemotherapy induction regimen.

Taken together, these data support an approach to intermediate-risk neuroblastoma that centers on moderately intensive chemotherapy and avoids radical surgery and external beam radiotherapy in the majority of patients. Therefore, the current COG intermediate-risk neuroblastoma strategy consists of combination chemotherapy including carboplatin, etoposide, cyclophosphamide, and doxorubicin. This is designed to test the hypothesis that this regimen will provide a greater than 90% EFS rate with minimal treatment-related morbidity. In addition, it builds on the previous CCG and POG experience by providing a longer duration of therapy for patients in the intermediate-risk category but with unfavorable biologic features (e.g., stage 4 infants with unfavorable Shimada pathology).

### *Treatment of High-Risk Disease*

Included in the high-risk disease group are the following patients: INSS stage 4 patients older than 1 year at diagnosis, any INSS stage 3 patient with amplified *MYCN*, INSS stage 3 patients older than 1 year at diagnosis with unfavorable Shimada pathology, INSS stage 2 patients with amplified *MYCN* and unfavorable Shimada pathology, and INSS Stage 4S patients with amplified *MYCN* (Table 31-9).

Historically, high-risk neuroblastoma patients have had long-term survival probabilities of less than 15%. [197,345,376,405,406,407,408,409,410,411,412,413,414,415,416,417,418 and 419] With the advent of comprehensive treatment approaches that include (a) intensive induction chemotherapy, (b) myeloablative consolidation therapy with stem cell rescue, and (c) targeted therapy for minimal residual disease, OS rates have improved ( Fig. 31-13). However, the current survival rates remain unacceptable and have come at the expense of significant immediate and long-term morbidity.

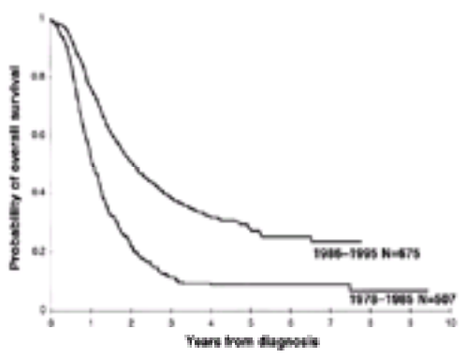



**FIGURE 31-13.** Improving survival for high-risk neuroblastoma patients. Comparison of 1,182 consecutive patients with stage 4 neuroblastoma diagnosed at age older than 1 year and treated at CCG institutions. (Redrawn with permission from Katherine Matthay, University of San Francisco, California.)

### Induction Therapy

The goal of induction therapy is to induce maximum reduction in tumor bulk at primary and metastatic sites. Neuroblastoma is typically sensitive to initial chemotherapy, even in cases with amplified *MYCN*. Retrospective analyses have shown an association of chemotherapy dose intensity with response and survival rates.[420,421] In particular, the dose intensity of the platinum compounds appears to correlate most significantly with disease outcome. [421] The appropriate duration of induction therapy is currently unknown, although it is generally agreed that it should be accomplished in as rapid a time frame as possible, before the acquisition of drug resistance.

The efficacy of induction chemotherapy regimens is assessed by the response rate, usually a combined measure of CR and PR and typically determined after a second surgical procedure. Although not definitively proven, there is a substantial body of evidence suggesting that the quality of remission at the end of induction chemotherapy is highly associated with long-term survival probability. [421,422,423,424,425,426,427 and 428] In a multivariate analysis performed with 549 high-risk neuroblastoma patients registered on the European Bone Marrow Transplantation Solid Tumor Registry, Ladenstein and colleagues [425] showed that persisting cortical bone lesions ($p$ = .004) and bone marrow involvement ($p$ = .03) were the only independent adverse prognostic factors. Thus, patients who achieve a CR or VGPR with induction chemotherapy have a better chance of cure, although some patients with a PR can be converted to CR with high-dose myeloablative therapy. [404,429]

Table 31-10 reviews the induction chemotherapy regimens used in the most recent cooperative group studies in the United States. It can be concluded from the U.S. experience that induction chemotherapy response rates were greatest in the regimens that contained higher overall dose intensities, especially in those with higher intensity of the platinum agents. For example, in POG 8742, regimen 1 contained almost twice the dose intensity of cisplatin and had nearly a 10% better induction response rate.[383] Future trials in the United States may further dose-intensify the platinum and alkylator components of induction therapy, although acute and long-term toxicities may limit this approach (see below).

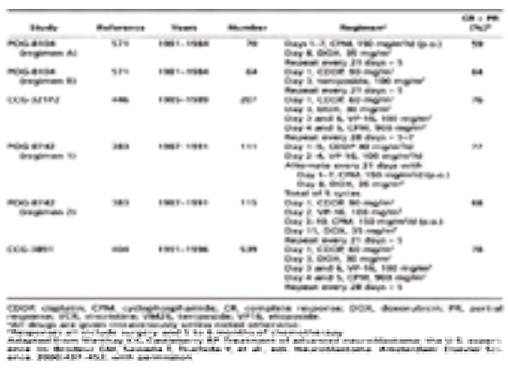


**TABLE 31-10. INDUCTION CHEMOTHERAPY REGIMENS AND RESPONSE RATES FROM RECENT PEDIATRIC ONCOLOGY GROUP AND CHILDREN'S CANCER GROUP COOPERATIVE GROUP TRIALS**

Experience from single-institution U.S. studies as well as multicenter studies in Japan and Europe suggest that alternate induction strategies may improve response rates and EFS. Kushner and colleagues[430,431] have reported that 21 of 24 patients achieved either CR or VGPR with an induction regimen that included a much higher dose intensity of cyclophosphamide, doxorubicin and cisplatin, but this may be associated with a significant risk of treatment-related leukemia. The Study Group of Japan for Advanced Neuroblastoma has reported a 92% CR + PR rate with six cycles of conventional doses of cyclophosphamide, vincristine, pirarubicin and cisplatin, although some of these patients may have progressed before consolidation therapy. [427] The ENSG has reported response rates between 69% and 96% for various induction regimens used in national trials. [428] Retrospective analysis showed a correlation between the cumulative drug doses and response rate. [428] The ENSG has directly tested the hypothesis that an increased dose intensity of induction chemotherapy improves response rate and survival in a recent randomized trial between two identical chemotherapy regimens that differed only in the rapidity of delivery [versus “rapid” COJEC (cyclophosphamide, vincristine [Oncovin], carboplatin [JM-8], etoposide [VP-16], and cisplatin)]. Results from this important trial should be available in the near future.

### Consolidation Therapy

The goal of consolidation therapy is to consolidate the response achieved during induction therapy by eliminating any remaining tumor, usually with myeloablative cytotoxic agents and stem cell rescue. The concept of eliminating resistant tumor clones with supralethal chemotherapy supported by ABMT has been actively investigated in neuroblastoma since the early 1980s. Since the first published trial of myeloablative consolidation therapy using high-dose melphalan alone, [432] multiple single-arm trials and registry reviews have been reported in the literature. [423,425,427,433,434,435,436,437,438,439,440,441,442,443,444,445,446,447,448,449,450,451 and 452] These studies are difficult to compare due to the heterogeneity of induction and consolidation regimens as well as varying strategies for stem cell harvesting and reinfusion. However, the majority of studies reported at least a trend toward improved survival probabilities compared to nonrandomized control groups and historical controls. The recent U.S. experience from trials involving 40 patients or more are listed in Table 31-11.

| Study | Reference | Number | Regimen[a] | 3-yr event-free survival[b] |
|---|---|---|---|---|
| POG-8340 | 349 | 81 | Melphalan, TBI | 38 |
| CCG-321P3 (Regimen 1) | 445 | 45 | Cisplatin, teniposide, doxorubicin, melphalan, TBI | 42 |
| CCG-321P3 (Regimen 2) | 445 | 54 | Cisplatin, etoposide, melphalan, TBI | 50 |
| CCG-321P3 (Regimen 3) | 445 | 48 | Carboplatin, etoposide, melphalan, TBI | 41 |
| CCG-3891 | 404 | 129 | Carboplatin, etoposide, melphalan, TBI | 43 |
| CCG-LA-6P | 572 | 77 | Carboplatin, etoposide, melphalan, local radiation | 62 |

TBI, total body irradiation.
[a]All studies used purged autologous bone marrow unless otherwise noted.
[b]Three-year event free survival rate from time of autologous marrow infusion.
[c]Ten percent of these patients received allogeneic bone marrow.
[d]This study was a CCG-sanctioned single-institution pilot study.
Adapted from Matthay KK, Castleberry RP. Treatment of advanced neuroblastoma: the U.S. experience. In: Brodeur GM, Sawada T, Tsuchida Y, et al., eds. Neuroblastoma. Amsterdam: Elsevier Science, 2000:437–452, with permission.

**TABLE 31-11. CONSOLIDATION REGIMENS AND 3-YEAR EVENT-FREE SURVIVAL RATES FROM TIME OF AUTOLOGOUS STEM CELL TRANSPLANTATION FROM RECENT PEDIATRIC ONCOLOGY GROUP AND CHILDREN'S CANCER GROUP COOPERATIVE GROUP TRIALS**

There have been two randomized studies of ABMT for high-risk neuroblastoma. The ENSG reported a trial of either high-dose melphalan myeloablation with autologous, unpurged bone marrow rescue versus no further therapy for patients who achieved a CR with induction chemotherapy. [453] Of 140 infants and children with disseminated disease, 95 achieved CR and 65 were randomized. The projected disease-free survival favored the transplanted group compared to the group stopping treatment (50% vs. 27% at 2 years; $p = .03$), although firm conclusions could not be drawn due to the small number of patients randomized. Matthay and colleagues [404] recently reported the results of a 5-year CCG study that tested the hypothesis that consolidation with myeloablative chemotherapy followed by purged autologous bone marrow rescue would improve survival probability compared to continuation chemotherapy. It is important to note that the continuation therapy was fairly intense but nonmyeloablative. A total of 539 eligible patients were enrolled onto this clinical trial designed with two sequential randomizations (± autologous marrow rescue and ± 13-*cis*-retinoic acid). A total of 379 patients were randomly assigned to autologous marrow rescue (n = 189) or continuation chemotherapy (n = 190). An intent-to-treat analysis showed a significant improvement in 3-year, EFS probability for the patients assigned to myeloablative therapy (34 ± 4% vs. 22 ± 4%; $p = .034$; Fig. 31-14A). Importantly, autologous transplantation appeared to have the largest impact on survival for the ultra-high-risk subset of patients, such as those with *MYCN* amplified metastatic disease diagnosed after 2 years of age. However, follow-up is relatively short, and these data will need further analysis due to the potential for late relapse in the transplant group.

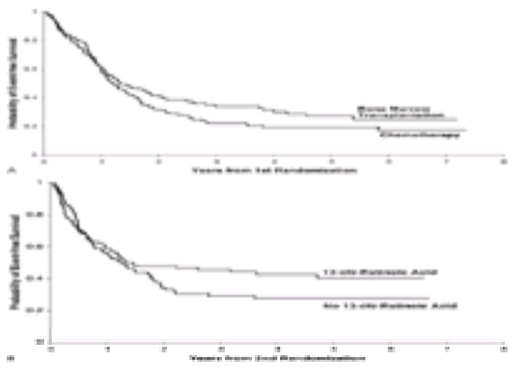


**FIGURE 31-14.** ABMT and 13-*cis*-retinoic acid improve event-free survival for high-risk neuroblastoma patients—results of CCG study 3891. [401] All patients were treated with identical induction chemotherapy and were first randomized to either purged ABMT or continuation chemotherapy, and then to either 13- *cis*-retinoic acid or no further therapy. **A:** Event-free survival for patients randomized to ABMT (n = 189) or intensive but nonmyeloablative continuation chemotherapy (n = 190) ( $p = .034$). **B:** Event-free survival from time of randomization to 13-*cis*-retinoic acid (n = 130) or no further therapy (n = 128) ($p = .027$). (Redrawn with permission from Dr. Katherine Matthay, University of San Francisco, California.)

It has recently become clear that using peripheral blood stem cells provides superior engraftment kinetics compared to conventional bone marrow grafts, and this technique might abrogate some transplant-related morbidity. [452,454] In addition, there is increasing experience with large-volume cytapheresis procedures in small children. [455] Last, there is the theoretical advantage that peripheral blood stem cell products are less likely to contain contaminating tumor cells, although Moss and colleagues [456] clearly demonstrated that circulating, clonogenic tumor cells may be present in patient peripheral blood samples obtained *at diagnosis*. Because peripheral blood stem cell harvests typically occur after two to four cycles of induction chemotherapy, and therefore follow an "*in vivo* purging," the majority of the pheresis products contains no contaminating tumor cells. [424,452,454] Thus, future clinical trials for high-risk neuroblastoma patients will most likely use autologous peripheral blood stem cells for myeloablative rescue.

The majority of high-risk neuroblastoma patients has metastases to the bone marrow at diagnosis. [231] Due to the unique expression of neural-specific antigens that are not normally present on the surface of hematopoietic cells, it is possible to detect submicroscopic neuroblastoma contamination of bone marrow (or peripheral blood) with monoclonal antibodies. [245,247] In addition, these neuroblastoma cells can be reliably depleted *ex vivo* using immunomagnetic purging. [458,459,460,461,462 and 463] There have been no randomized trials designed specifically to investigate the impact of *ex vivo* tumor cell purging of stem cell products on the outcome of high-risk neuroblastoma patients. However, there has been direct demonstration that contaminating neuroblastoma cells in autologous marrow samples contributed to disease relapse in three patients who received unpurged marrow infusions. [464] The recent randomized trial conducted by CCG for high-risk neuroblastoma patients that showed an advantage to myeloablative therapy compared to continuation chemotherapy used purged autologous marrow. [404] Thus, although most investigators agree that *ex vivo* immunomagnetic purging of bone marrow products is both safe and effective in depleting contaminating tumor cells, it is unclear what impact, if any, purging may have on peripheral blood stem cell products obtained after effective *in vivo* purging. A randomized clinical trial designed to test the efficacy of *ex vivo* purging of immunocytochemically negative peripheral blood stem cell products has recently been initiated by the COG.

In two relatively small studies, no evidence for a "graft-versus-tumor" effect could be found for high-risk neuroblastoma patients receiving allogeneic marrow transplants. A nonrandomized study was conducted by CCG comparing 20 patients receiving allogeneic marrows to 36 children undergoing ABMT after marrow purging. [442] The estimated progression-free survival at 4 years was 25% for the allogeneic group versus 49% for the autologous group ( $p = .51$). In a similar study from the European Group for Bone Marrow Transplantation, [441] the 2-year progression-free survival for 17 allogeneic transplants (35%) was not significantly different from that of 34 autologous transplants (41%). Thus, there is no evidence to support use of an allogeneic source of stem cells.

The potential that two myeloablative consolidations might be better than one is currently being investigated. The main theoretical advantage of a tandem transplant approach involves providing multiple non–cross-resistant agents at maximal doses in a relatively rapid sequence. Philip and colleagues [440] demonstrated that this approach, when supported by purged, autologous bone marrow, was associated with significant transplant-related morbidity and mortality, but also with an encouraging 5-year survival of 32% in a cohort of 33 very-high-risk patients with relapsed or refractory disease. More recently, Grupp and colleagues [454] showed that a tandem myeloablative consolidation with peripheral blood stem cell support was feasible with rapid myeloid engraftment in a limited institution pilot study of 39 patients. Transplant-related mortality was 8% (3 of 39) and the 3-year EFS was estimated at 58%. A randomized trial of single versus tandem myeloablative consolidations is necessary to determine the true impact of a tandem high-dose myeloablative consolidation strategy.

**Minimal Residual Disease Therapy**

The goal of this phase of therapy is to eradicate any residual tumor cells using agents that are typically not directly cytotoxic and therefore theoretically active against highly chemoresistant minimal residual disease.

Despite the improvements in induction chemotherapy response rates and efficacy of myeloablative consolidation procedures, the majority of high-risk neuroblastoma

patients experiences disease relapse. This is often despite the fact that many patients reach the end of consolidation therapy with no disease detectable by conventional methodologies. It is therefore assumed that microscopic residual disease is often present after myeloablative consolidation. In addition, cell lines derived from relapse specimens have been shown to be highly chemoresistant,[465] suggesting that selection for, or acquisition of, drug resistance are the major mechanisms by which these tumor cells survive intensive induction and consolidation therapies.

Several novel agents specifically targeted to the unique biology of neuroblastoma may be effective for eliminating minimal residual disease. The retinoids are a class of compounds that have been known for two decades to induce cellular differentiation with a concomitant decrease in proliferation of neuroblastoma cells *in vitro*.[466] A phase I trial of 13-*cis*-retinoic acid in neuroblastoma patients with any disease status after myeloablative consolidation defined a maximum tolerated dose of 160 mg per $m^2$ per day given on a twice-daily schedule.[467] Of ten patients with measurable disease at study entry, three had complete clearing of bone marrow metastases. The efficacy of 13-*cis*-retinoic acid was recently tested in a randomized trial using a factorial design following a randomization to myeloablative or continuation chemotherapy (see above).[404] A total of 130 patients were randomized to receive either six cycles of 160 mg per $m^2$ per day divided twice-daily schedule for 2 weeks per month or no further therapy. The cohort of patients assigned to receive posttransplant therapy with 13- *cis*-retinoic acid had a significantly improved EFS probability (46 ± 6% versus 29 ± 6% from second randomization; $p = .027$; Fig. 31-14B). Other retinoids such as 9-*cis*-retinoic acid, all-*trans*-retinoic acid, or fenretinide may also have activity for minimal residual disease in high-risk neuroblastoma patients.

An alternative strategy that appears promising for application during the minimal residual disease phase of therapy is targeted molecules directed against neuroblastoma-specific cellular antigens. Murine, chimeric, and humanized antibodies specific to the cell surface ganglioside $G_{D2}$, either alone or with cytokines, have shown activity in preclinical models[459,468,469,470 and 471] as well as phase I[472,473,474,475,476,477 and 478] and phase II[479,480] clinical trials. The murine monoclonal antibodies 3F8[468] and GD2a,[477] and the human-mouse chimeric monoclonal antibody ch14.18[473] have received the most clinical attention. Measurable responses have been observed in refractory neuroblastoma patients[474,475,480] justifying further examination in phase III clinical trials. Indeed, some investigators have suggested that antibody therapy alone may be effective to consolidate induction remission and may obviate the need for myeloablative chemoradiotherapy.[480] Limiting the broad application of this strategy are difficulties with antibody production and the fact that the majority of patients develops neutralizing antibodies, even to the chimeric molecules.[474,475,480] Neuropathic pain is also a significant immediate toxicity and can be dose limiting.[474,475 and 476,480] A randomized clinical trial designed to test the efficacy of ch14.18 with interleukin-2 and granulocyte-macrophage colony-stimulating factor versus no antibody/cytokine therapy after myeloablative chemotherapy is planned within the COG.

The appropriate methodologies to detect minimal residual disease are not yet decided. For residual cortical bone disease, MIBG may improve the sensitivity for detecting active disease, but may need to be combined with conventional bone scintigraphy.[475,481] Immunocytochemical analysis of bone marrow aspirates with a panel of monoclonal antibodies increases the sensitivity of detection to at least 1:100,000 nucleated cells and may be of prognostic value.[266] Additional sensitivity, perhaps greater than 1:1,000,000 nucleated cells, may be added with reverse transcriptase-polymerase chain reaction methodologies by targeting the expression of one or several tumor-specific messages such as tyrosine hydroxylase, PGP 9.5, GAGE, or MAGE.[482,483 and 484] However, the clinical significance of positivity at this level of sensitivity remains to be determined.

## TREATMENT OF RECURRENT DISEASE

A variety of approaches are available to treat patients with recurrent or refractory neuroblastoma. However, the ability to cure patients who recur after treatment for high-risk disease remains very low. Current innovative approaches include novel cytotoxic agents, targeted delivery of radionuclides, retinoids, and immune-mediated therapy.

### Novel Cytotoxic Agents

Topotecan, a topoisomerase I inhibitor, is a new agent that is currently in phase II trials and does not require dose-intensification for efficacy.[485,486] This agent is being evaluated alone or in combination with cyclophosphamide in an intergroup trial in POG and CCG (now COG), and early results are promising.[384,487,488 and 489] Other novel chemotherapeutic agents under investigation in selected institutions include paclitaxel (Taxol), irinotecan, and rebeccamycin.[384,490,491] Oral etoposide has also been used mainly as a palliative agent, and it is well tolerated.[492] A recent trial includes escalating doses of melphalan with BSO, a highly specific GSH inhibitor. BSO is designed to overcome alkylator resistance mediated through GSH-dependent alkylator efflux by depleting the cells of GSH.[493,494] This combination is a promising approach to the treatment of neuroblastoma, particularly in the context of marrow ablation with stem cell rescue.

### Targeted Delivery of Radionuclides

There are several approaches to targeted delivery of radiation to neuroblastoma cells. These include the attachment of radionuclides to MIBG,[495,496,497,498,499 and 500] to SS analogs,[90,501,502,503 and 504] or to anti-$G_{D2}$ antibodies.[474,475,480,505,506,507 and 508] Radioactive MIBG is used in some frontline therapy for high-risk neuroblastoma in Europe, but it is not in wide use in the United States except for recurrent or refractory disease, with or without stem cell rescue. Future trials may combine targeted radionuclides with conventional cytotoxic chemotherapy.

### Retinoid Compounds

Treatment with 13-*cis*-retinoic acid was not very effective against bulk disease, but it has proven to be an effective treatment in states of minimal residual disease.[404] Newer retinoids are being developed for use in neuroblastoma treatment, and fenretinide is currently the most attractive agent.[509,510,511 and 512] Unlike retinoic acid, which can induce differentiation of neuroblastoma cells in culture, fenretinide appears to primarily induce apoptosis, even in neuroblastoma cells that are resistant to 13-*cis*-retinoic acid.

### Immune-Mediated Therapy

Neuroblastoma can be treated with unlabeled antibody against the ganglioside $G_{D2}$. This antibody may have direct cytotoxic effects as well as induce antibody-dependent cellular cytotoxicity.[480,513] In addition, vaccine trials are being conducted that attempt to induce humoral and cellular responses directed to neuroblastomas.[514,515,516 and 517]

## COMPLICATIONS OF NEUROBLASTOMA AND ITS TREATMENT

A variety of complications of neuroblastoma and its treatment may occur that are not particularly unique to this tumor. These include late effects of chemotherapy, radiation therapy, and surgery.[518] Patients in the low-risk category will likely experience only the consequences of surgery, and because their prognosis is excellent, aggressive surgery should be minimized. Patients in the intermediate-risk category are exposed to surgery as well as moderately intensive chemotherapy. However, the overall regimen is short and well tolerated, and various pairs and combinations of drugs are used to minimize the likelihood of significant side effects of any one agent.

High-risk patients are at the greatest risk of experiencing complications of treatment. There are problems with linear growth, cardiac function, renal function, and other organs.[518] In addition, ototoxicity has been a particular problem from the use of platinum-containing regimens to treat very young children.[519] The extensive use of alkylating agents and topoisomerase II inhibitors results in a higher prevalence of sterility as well as second malignancies, particularly myelodysplastic syndromes and acute nonlymphoblastic leukemias.[431,518] However, the latter problem may be particularly regimen and schedule dependent.

Indeed, a number of different second neoplasms have been reported in patients with neuroblastoma after treatment, such as thyroid cancer, pheochromocytoma, brain tumors, acute leukemia, osteosarcoma, breast cancer, and renal cell carcinoma.[520,521,522,523,524,525,526,527 and 528] None of these second cancers has occurred with sufficient frequency to indicate a specific relationship between neuroblastoma and any other neoplasm.[529] Furthermore, patients with neuroblastoma do not appear to be at increased risk for developing specific second cancers other than the risk associated with certain chemotherapeutic agents or radiation therapy.

## NEUROBLASTOMA SCREENING STUDIES

One approach to improve the long-term outcome of patients with neuroblastoma is to identify patients earlier in the course of their disease. This assumes that (a) patients with more advanced stages of disease, and thus a higher risk of treatment failure, “progress” from more localized disease over time; and (b) that there is in interval during which they could be detected before progression and clinical presentation. Because neuroblastomas frequently produce increased levels of catecholamines whose metabolites are detectable in the urine, mass screening of infants for neuroblastoma has been investigated. Indeed, such a screening program has been ongoing in Japan for about 25 years.[530,531,532,533,534 and 535] Efforts have also been undertaken in North America and Europe to answer questions concerning the feasibility and utility of mass screening for neuroblastoma.[61,62,536,537,538,539,540 and 541]

Studies of the clinical and cytogenetic features of tumors identified as a result of mass screening of infants for neuroblastomas suggest that the majority of patients identified has lower stages of disease, and virtually all of the tumors are biologically favorable, with a DI in the hyperdiploid or near-triploid range and with a normal *MYCN* copy number.[533,542,543,544 and 545] Previous studies have demonstrated that such findings are generally associated with a very favorable outcome. Therefore, the results of the screening studies have suggested at least two possibilities: (a) that all neuroblastomas begin as tumors with a more favorable genotype and phenotype, and some evolve into more aggressive tumors with adverse genetic features; or (b) there are at least two different subsets of neuroblastoma, and the more favorable type presents earlier and therefore is the predominant type detected by screening. Current evidence is more consistent with the latter explanation and suggests that neuroblastomas detected by screening are predominantly of the most favorable biologic type (type 1; Table 31-2).[24,103,109]

In addition to the genetic data discussed above, the accumulating evidence suggests that the prevalence of neuroblastoma in screened populations is increased by 50% to 100% over that seen in unscreened populations, and that the prevalence and mortality of neuroblastoma in patients older than 1 year has not changed appreciably.[61,62 and 63,538,541] Taken together with the biologic information, this suggests that screening is detecting tumors in a substantial number of patients who likely would never develop symptomatic disease because their tumors would have regressed or matured without therapy. Many of the tumors detected by screening in patients aged 6 months have favorable biologic features [545] and could be cured easily with surgery or relatively mild therapy. Several studies in Japan and Europe are screening patients at a later time, such as at 8 to 14 months.[63,536,537,539,540,546] It appears that the prevalence of “overdiagnosis” by screening at a later time is lessened, and there is a greater frequency of patients with unfavorable clinical and biologic features. However, it remains to be determined whether early detection by screening will improve the outcome of patients whose tumors have unfavorable biologic features.

There have been several reports of the incidental prenatal detection of neuroblastoma by maternal ultrasound.[547,548,549 and 550] The majority of these patients has had cystic adrenal tumors with favorable biologic markers and an excellent outcome. However, a few have rapid tumor progression shortly after birth, especially those with extensive liver metastases. Because the quality of maternal ultrasound has improved and it is used more frequently, it is likely that more cases will be diagnosed. However, unless there is massive liver involvement or unfavorable biologic features (DI = 1, *MYCN* amplification), such cases can probably be managed conservatively and chemotherapy or radiotherapy can be avoided.

## FUTURE CONSIDERATIONS

Short-term improvements in the outcome of neuroblastoma patients are likely to come from more effective use of existing modalities, including newer agents (topotecan, fenretinide) as well as stem cell support for high-risk patients. Furthermore, the use and continued improvement of risk stratification schema should allow the most appropriate intensity of therapy to be selected, minimizing the likelihood of undertreatment or overtreatment. However, longer-term improvement in the management of neuroblastoma patients are likely to come from several other areas. These include (a) the identification of individuals with a genetic predisposition to develop this disease, (b) additional tumor markers to follow tumor response to treatment, (c) better biologic characterization of tumors for classification and prognostication, and (d) biologically directed therapy that would presumably be more effective and less toxic than current regimens.

### Neuroblastoma Predisposition

As improvements occur in the long-term outcome of neuroblastoma patients, it will become increasingly important to identify individuals who are predisposed to develop this tumor. This would be useful to determine risk for siblings of neuroblastoma patients and would provide useful information for genetic counseling of patients and their offspring. Maris and colleagues [48] have recently identified, using linkage analysis, a locus on 16p that may be responsible for the majority of hereditary neuroblastomas. Although a gene has not been identified yet, DNA polymorphisms closely linked to this predisposition locus could be used to identify predisposed individuals in informative families. Further analysis should determine if there are additional predisposition loci.

### Tumor Markers

Following the levels of urinary catecholamine metabolites of patients with neuroblastoma is not as sensitive as a-fetoprotein or b-human chorionic gonadotropin for following germ cell tumors. Measurement of several candidate serum markers has been proposed, including ferritin, NSE, $G_{D2}$, chromogranin A, and others, but none has yet emerged as sufficiently sensitive or specific to be useful in this context. As more is understood about the biology of neuroblastomas, additional secreted neural-specific proteins may be identified that could be used to follow response to treatment and to predict early relapse. Such markers might obviate the need for multiple diagnostic imaging studies and marrow sampling in patients both on and off therapy.

### Biologic Characterization of Tumors

Biologic markers such as DI, *MYCN* amplification, 1p deletion, unbalanced 17q gain, *TRKA* expression, and others have proven to be powerful prognostic variables. However, as the genetic and biologic complexity of neuroblastoma becomes better understood, it is likely that additional markers of low-, intermediate- and high-risk disease will be identified. It is likely that these additional biologic variables will replace such clinical distinctions as age and stage in determining the most appropriate type and intensity of treatment to optimize cure and minimize unnecessary side effects. Indeed, newer techniques such as microarray analysis of the genomic changes or expression profile of tumors may allow many variables to be analyzed simultaneously, providing a comprehensive genetic picture of each patient's tumor.[551,552,553 and 554]

### Future Treatment Strategies

#### *Anti-Angiogenesis Therapies*

In neuroblastoma, high-risk disease features are correlated with tumor vascularity, suggesting that angiogenesis inhibitors may be a useful addition to current therapeutic strategies.[555,556] Furthermore, there is evidence that angiogenic factors may be expressed at higher levels in more advanced stage or biologically unfavorable neuroblastomas.[557,558 and 559] Several groups have examined the efficacy of TNP-470 (TAP Pharmaceuticals, Deerfield, IL) in human and murine neuroblastoma xenograft models.[560,561,562 and 563] In general, TNP-470 inhibited the development of tumors or the growth rate of small tumors, and response was related to tumor burden. TNP-470 also significantly inhibited tumorigenicity when administered after cyclophosphamide.[561] These data show that TNP-470 is a promising inhibitor of human neuroblastoma growth, suggesting that it may be a useful adjunct for high-risk neuroblastoma patients. In addition, therapy aimed at integrins or vascular endothelial growth factor receptors may also be valuable approaches to inhibit angiogenesis of neuroblastomas.[564,565 and 566]

#### *Tyrosine Kinase Inhibitor Therapies*

Neuroblastomas frequently express Trk family tyrosine kinase receptors, which in turn regulate growth, differentiation, and cell death. CEP-751 (KT-6587), an indolocarbazole derivative, is an inhibitor of Trk family tyrosine kinases at nanomolar concentrations. Evans and colleagues [567] showed a significant growth inhibitory effect of CEP-751 on several human neuroblastoma cell lines growing as xenografts. Furthermore, inhibition of growth was greater in the SY5Y cell line transfected with *TRKB* compared to the untransfected parent cell line expressing no detectable *TRKB*. Finally, there was no apparent toxicity identified in any of the treated mice. These results suggest that CEP-751 or other TRK-specific kinase inhibitors may be useful therapeutic agents for neuroblastomas.

### *Induction of Differentiation or Cell Death*

Treatment with 13-*cis*-retinoic acid to induce differentiation appears to be effective in the setting of minimal residual disease. [404] Currently, no other agents are being used to induce neuroblastoma differentiation, but small peptides or molecules that are agonists of the *TRKA*/NGF pathway potentially would induce differentiation. Conversely, antagonists of *TRKA* or *TRKB* may induce programmed cell death by blocking critical survival pathways. In this regard, fenretinide, a retinoid that induces apoptosis in neuroblastoma cells, is being developed for treatment of high-risk patients, [509,510,511 and 512] and newer retinoids to induce differentiation of cell death are being investigated. Finally, betulinic acid is a novel agent that induces apoptosis by triggering release of pro-apoptotic factors from mitochondria, and this agent may have therapeutic value in neuroblastomas. [568,569 and 570]

In summary, considerable progress has been made in understanding the genetic basis of clinical heterogeneity in neuroblastomas. Tumors at diagnosis can be more accurately categorized as having a low, intermediate, or high risk of relapse, and this has resulted in less overtreatment or undertreatment than when therapy was based on clinical variables alone. Improvements have also occurred in the cure rates of these patients with more judicious use of conventional therapeutic agents, with or without cytokine support or stem cell rescue. The real challenge is to translate our new insights concerning the biology of this disease into novel approaches to treatment that target critical pathways involved in tumor cell survival or maintenance of the malignant state. It is encouraging that we are already beginning to meet this challenge, so the prospects for the future look promising.

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## ABBREVIATIONS

| | |
|---|---|
| ABMT | Autologous bone marrow transplantation |
| BDNF | Brain-derived neurotrophic factor |
| BSO | Buthionine sulfoximine |
| CCG | Children's Cancer Group |
| CDDP | Cisplatin |
| COG | Children's Oncology Group |
| CPM | Cyclophosphamide |
| CGH | Comparative genomic hybridization |
| CR | Complete response |
| CT | Computed tomography |
| DI | DNA index |
| dmin | Double-minute chromosome |
| DOPA | 3,4-dihydroxyphenylalanine |
| DOX | Doxorubicin |
| EFS | Event-free survival |
| ENSG | European Neuroblastoma Study Group |
| GSH | Glutathione synthetase |
| HSR | Homogeneously staining region |
| HVA | Homovanillic acid |
| INSS | International Neuroblastoma Staging System |
| kb | Kilobase |
| LDH | Lactate dehydrogenase |
| LOH | Loss of heterozygosity |
| MIBG | Metaiodobenzylguanidine |
| MKI | Mitosis-karyorrhexis index |
| MRI | Magnetic resonance imaging |
| NF1 | Neurofibromatosis type 1 |
| NGF | Nerve growth factor |
| NSE | Neuron-specific enolase |
| OPEC | Vincristine (Oncovin), cisplatin, etoposide, cyclophosphamide |
| OS | Overall survival |
| POG | Pediatric Oncology Group |
| PR | Partial response |
| SS | Somatostatin |
| TNM | Tumor-node-metastasis |
| TRK | Tyrosine kinase receptor |
| TRKA | Nerve growth factor receptor |
| VGPR | Very good partial response |
| VIP | Vasoactive intestinal peptide |
| VMA | Vanillylmandelic acid |
| VP-16 | Etoposide |

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# RHABDOMYOSARCOMA AND THE UNDIFFERENTIATED SARCOMAS

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## INTRODUCTION

A malignant tumor of mesenchymal cell origin is called a *sarcoma*. Mesenchymal cells normally mature into skeletal muscle, smooth muscle, fat, fibrous tissue, bone, and cartilage. Rhabdomyosarcoma (RMS) is believed to arise from immature mesenchymal cells that are committed to skeletal muscle lineage, but these tumors can arise in tissues in which striated muscle is not normally found, such as in the urinary bladder. Undifferentiated sarcomas are mesenchymally derived tumors that cannot be ascribed to any specific lineage. Some tumors also may display multilineage markers, including ectomesenchymomas, which are tumors with evidence of both skeletal muscle and neuronal lineage, and malignant Triton tumors, which are malignant peripheral nerve sheath tumors (schwannomas) with evidence of rhabdomyoblastic elements.

The incidence of RMS is slightly less than one-half that of all other forms of non-RMS soft tissue sarcoma (NRSTS) combined. Important epidemiologic, biologic, and treatment differences exist both within the family of RMS and between RMS and NRSTS. Although RMS traditionally has been staged by a unique surgicopathologic staging system [the clinical group (CG) system], a recent move has been made to adopt a more uniform, modified (site-based) tumor-node-metastasis (TNM) staging system comparable to what has been used for adult NRSTS. The development of increasingly intensive, large-scale, international, collaborative, multimodality therapeutic protocols for treating these tumors, particularly the Intergroup Rhabdomyosarcoma Studies (IRS), has led to a steady improvement in the curability of these neoplasms, especially for the group of patients with locally extensive but unresectable tumors. Along with the improvements in outcome, however, have come increases in both short- and long-term sequelae of therapy.

## EPIDEMIOLOGY AND GENETICS

The annual incidence of RMS in children 20 years of age or younger is 4.3 cases per million children, with approximately 350 new cases diagnosed in the United States each year.[1] Among the extracranial solid tumors of childhood, RMS is the third most common neoplasm after neuroblastoma and Wilms' tumor. Almost two-thirds of cases of RMS are diagnosed in children aged 6 years or younger, with a smaller incidence peak in early-midadolescence. The tumor is slightly more common in boys and males (11.8 per million) than in girls and females (10.3 per million).[1] An international study confirmed previous reports of racial and gender differences in the incidence of RMS.[2] In the United States, the incidence of RMS for black females was found to be only one-half that for white females, whereas the rate for males was similar in both ethnic groups. The incidence of RMS in most of Asia appears to be lower than among mainly white populations in Western industrialized countries, confirming an earlier finding of a lower relative frequency of RMS in children of South Asian ethnic origin residing in Britain.[3]

Although these tumors may arise virtually anywhere in the body, certain distinctive clusters of features emerge regarding age at diagnosis, site of primary tumor, and histology. For example, head and neck tumors are most common in children younger than 8 years and, if arising in the orbit, almost always are of the embryonal variety. On the other hand, extremity tumors are seen more commonly in adolescents and are more frequently of the alveolar subtype. A unique form of RMS arising from the bladder or vagina—the botryoid variant (so named because of its resemblance to a protruding cluster of grapes)—is seen almost exclusively in infants.

Until recently, published studies of potential etiologic factors related to the development of RMS were composed of relatively small series. Although the overwhelming majority of cases of RMS occur sporadically, the development of RMS has been associated with certain familial syndromes, such as neurofibromatosis and the Li-Fraumeni syndrome (LFS), which includes familial clustering of RMS and other soft tissue tumors in children, with adrenocortical carcinoma and early-onset breast carcinoma in adult relatives.[4,5] The LFS has been associated with germline mutations of the p53 tumor suppressor gene.[6] In a study of 33 cases of sporadic RMS, 3 of 13 children younger than 3 years at diagnosis (as compared with none of the 20 children older than 3 years) were found to have germline mutations in their p53 gene.[7] This finding suggests that at least some very young children with seemingly sporadic RMS may have a hereditary predisposition to cancer or, possibly, an increased susceptibility to potentially toxic environmental agents (discussed later). It also raises the difficult ethical questions of whether family members of children younger than 3 years of age with a diagnosis of RMS may benefit from cancer risk screening,[8] and whether children with germline p53 mutations should have their therapy altered to minimize or reduce their exposures to potentially carcinogenic interventions (e.g., ionizing radiation, alkylating agents, epipodophyllotoxins).

The overall risk of a genetic predisposition to cancer has been estimated to be 7% to 33%, a range based on the patterns of cancer in the families of 151 children with soft tissue sarcoma.[9] These tumors included syndromes that appeared to be independent of p53 abnormalities. Of further interest, RMS has been observed in association with the Beckwith-Wiedemann syndrome, a fetal overgrowth syndrome marked by abnormalities on 11p15, where the insulin-like growth factor-2 (IGF-2) gene is located.[10] Some factors that may play a role in these phenomena are discussed later.

In a study of fetal loss and infant deaths in families of children with soft tissue sarcomas (two-thirds of which were RMS), 50 of the 157 families were classified as being genetically predisposed to cancer; one-third of these families had either classic LFS or a variant of LFS.[11] Reproductive loss was not related to index histology but was significantly higher in families with genetic disease than in those with sporadic disease, with most of the excess risk concentrated among the families affected by neurofibromatosis. Results of a large, national case-control study were reported in 1993; this study involved 322 RMS patients younger than 20 years and enrolled in the third IRS study (IRS-III) and an equal number of randomly selected age-, gender-, and race-matched controls.[12] Use of marijuana by a mother in the year before a child's birth was associated with a threefold increased risk of RMS in the child, and maternal cocaine use was associated with a fivefold increased risk. Use of marijuana, cocaine, or any recreational drug by a father also was associated with an approximately twofold increased risk. Consistent with a potential interaction between genetic susceptibility (e.g., germline p53 mutations) and environmental factors in the development of some cases of RMS, use of cocaine by the mother and

use of marijuana by both parents were associated with a significantly earlier age at diagnosis of RMS (as compared with that of all children in IRS-III).

## MOLECULAR BIOLOGY

Multiple molecular genetic alterations involving both muscle differentiation pathways and cell proliferation pathways appear likely to lead to the development of RMS. As the details of these pathways become better defined, lesions at any point within a given pathway likely will have similar consequences; thus, an entire pathway will have to be evaluated rather than looking at an isolated genetic alteration. For example, if alterations in the pRB pathway are common in RMS, p16, pRB, and CDK4 all would have to be considered as targets within this pathway. Much has been learned in the last decade regarding the specific molecular genetic alterations associated with the development of this tumor. In this section, known genetic alterations that occur in RMS are reviewed, as are the alterations in growth factors, oncogenes, and tumor suppressor genes that have been described in these tumors.

The two major histologic subtypes of RMS—embryonal (ERMS) and alveolar (ARMS)—have been found to have characteristic but distinct genetic alterations presumed to play a role in the pathogenesis of these tumors. ARMS has been demonstrated to have a characteristic translocation between the long arm of chromosome 2 and the long arm of chromosome 13, designated in shorthand notation as t(2;13)(q35;q14).[13,14] This translocation has been cloned molecularly and has been shown to involve the juxtaposition of the PAX3 gene (or, rarely, the PAX7 gene located at chromosome 1p36), believed to regulate transcription during early neuromuscular development, and the FKHR gene, a member of the forkhead family of transcription factors.[15,16] Presumably, the consequence of this fusion transcription factor is the abnormal activation of transcription from a gene or genes that contribute to the transformed phenotype. Although the precise consequence of this tumor-specific translocation remains to be elucidated, cDNA microarray analysis has shown that the PAX-FKHR fusion expressed in fibroblasts specifically turns on an array of myogenic factors.[17] Furthermore,PAX3-FKHR has been found to up-regulate c-MET expression, a receptor tyrosine kinase that has been implicated in transformation.[18] The polymerase chain reaction is likely to be used more widely in the near future for precise confirmation of the diagnosis of ARMS on the basis of genetics. Recently, a novel amplicon has been identified at 13q31 in approximately 20% of cases of ARMS, suggesting that one or more genes at this locus contribute to the pathogenesis of these tumors.[19]

The other major histologic subtype—ERMS—is known to have loss of heterozygosity (LOH) at the 11p15 locus.[20,21] Furthermore, this LOH has been shown to involve loss of maternal genetic information with duplication of paternal genetic material at this locus.[22] This region is of particular interest because it is the location of the IGF-2 gene, which codes for a growth factor believed to play a role in the pathogenesis of RMS (discussed later). IGF-2 has been demonstrated to be imprinted with only the paternal allele being transcriptionally active.[23,24] Therefore, it is conceivable that in this tumor, LOH with paternal disomy may lead to overexpression of IGF-2. However, also possible is that LOH at 11p15 may reflect the loss of a tumor suppressor activity that has not been identified or that both activation of IGF-2 and loss of tumor suppressor activity result from LOH at 11p15 in ERMS.[25]

One recent pilot study used comparative genomic hybridization and fluorescent *in situ* hybridization to identify novel genomic imbalances in patients with ERMS.[26] One particularly interesting area of loss was identified at 9q22, corresponding to the location of a presumed tumor suppressor gene that has been implicated in RMS genesis in a murine model of Gorlin syndrome (see the section Animal Models).

Several preliminary reports in the last decade suggested that ploidy (the number of chromosomes present in the tumor cell, generally measured by flow cytometry) was associated favorably with prognosis in patients with ERMS.[27,28] Specifically, diploid tumors (tumors with 46 chromosomes) were thought to have a prognosis worse than that of hyperdiploid tumors (tumors with more than 51 chromosomes); however, additional data supporting these initial observations have not been forthcoming.

Both ARMS and ERMS appear to overproduce IGF-2, a growth factor that has been shown to stimulate the growth of these tumor cells.[29] Blocking monoclonal antibodies directed against the receptor for IGF-2 (type 1 IGF receptor) have been demonstrated to inhibit growth of RMS both *in vitro* and *in vivo*.[29,30] Therefore, IGF-2 appears likely to play an important role in the unregulated growth of these tumors. The mechanism that leads to overproduction of IGF-2 in these tumors is unclear, although loss of imprinting of this locus has been implicated as one potential mechanism of IGF-2 overexpression.[31] Normal tissue, including fetal muscle, expresses IGF-2 only from the paternal allele; several cases of both ARMS and ERMS have shown expression from both parental alleles, a phenomenon termed *loss of imprinting*. The mechanisms involved in normal imprinting and the abnormalities that lead to loss of imprinting currently are the subject of much investigation.

Of further note, PAX3/FKHR expression can increase both IGF-2 expression and an IGF-binding protein, IGFBP-5.[17] The p53 tumor suppressor gene pathway has been implicated also in RMS. RMS tumors and cell lines evaluated have had loss-of-function p53 mutations that were diverse in nature.[32] Of interest is the finding of a codon-248 mutation in one RMS cell line, because a mutation at this codon has been observed also as a germline mutation in a case of LFS in which the index case was an RMS. Not known, however, is whether alterations in p53 function are primary events in the pathogenesis of these tumors or whether these alterations are associated more often with progression events. The true frequency of p53 mutations in RMS is unclear, but it appears to be relatively common.[33,34] As noted, to discern truly the role of p53 alterations in RMS, one needs to evaluate other molecules involved in p53 regulation. The two most prominent regulators of p53 function are MDM2 and $p19^{ARF}$. MDM2 negatively regulates p53, and this gene has been found to be amplified in RMS.[35,36 and 37] Another protein, $p19^{ARF}$, negatively regulates MDM2; thus, loss of $p19^{ARF}$ could be equivalent to MDM2 amplification or p53 loss. To date, the status of $p19^{ARF}$ has not been systematically evaluated in RMS.

The oncogene abnormalities observed most frequently in RMS are RAS mutations. Activated forms of both N-RAS and Kirsten-RAS (K-RAS) have been isolated from both RMS cell lines and tumor specimens.[38,39] A survey of ERMS tumor specimens found a 35% incidence of either activated N-RAS or K-RAS.[40] As with the p53 tumor suppressor gene, whether these alterations are involved primarily in the pathogenesis of these tumors or reflect secondary abnormalities that occur during progression events is not known. HER-2/neu gene amplification has not been studied well in RMS; only one of nine cases studied was demonstrated to have gene amplification in the one report published to date.[41]

The discovery of the myogenic basic helix-loop-helix (bHLH) MyoD family of proteins (including MyoD, myogenin, myf5, and MRF4) has greatly enhanced understanding of normal skeletal muscle differentiation.[42] These proteins function to commit mesenchymal cells to a skeletal muscle lineage and to activate their terminal differentiation program by inducing transcription of skeletal muscle–specific proteins, such as myosin and creatine kinase. The almost universal expression of MyoD family proteins in RMS provides further strong evidence of the skeletal muscle lineage of these tumors and has allowed for further refinement in the classification of pediatric sarcomas. For example, some cases that previously would have been called *undifferentiated sarcomas* can be classified as RMS on the basis of expression of these lineage-specific transcription factors.

However, the failure of RMS cells to differentiate terminally and the growth arrest that occurs despite the expression of these bHLH proteins has raised the question of what is altered in this pathway as compared with normal skeletal muscle cells. Initial studies aimed at answering this question suggested that RMS cells may lack a factor necessary for MyoD activity.[43] More recently, several investigators have suggested that MyoD may activate expression of p21, a cyclin-dependent kinase inhibitor that appears to function as a cell cycle arrest signal.[44,45 and 46] One possibility is that in normal skeletal muscle, bHLH proteins activate expression of p21, which leads to cell cycle exit, and also activate the expression of skeletal muscle–specific proteins, whereas some component of this pathway is defective in RMS cells, leading to continuous cell cycling despite expression of muscle-specific proteins. However, examination of p21 in 12 RMS tumor specimens failed to reveal the presence of mutations on either the coding region or promoter region of this gene.[47]

Abnormalities in the Rb pathway also have been implicated in RMS. In addition to RB, this pathway involves CDK-4/6, $G_1$ cyclins, and $p16^{INK4a}$. Both pRB and CDK-4 have been shown to be involved directly in normal myogenesis.[48,49,50,51 and 52] Although no mutations in RB have been demonstrated in RMS,[40,53] CDK4 has been shown to be amplified in RMS cell lines,[54] and loss of $p16^{INK4a}$ has been seen also in RMS cell lines and tumors.[55]

## ANIMAL MODELS

Several genetic models of RMS were created recently in mice. An earlier report demonstrated that SV40 T-antigen transgene, which can inactivate both p53 and pRB, led to the development of RMS at various anatomic sites.[56] More recently, a heterozygous knockout mouse lacking the PTC gene was shown to develop RMS at high frequency.[57] This is interesting, because the PTC gene is known to function as the receptor for Sonic Hedgehog, a known mediator of early skeletal muscle development.[58] Finally, a combined transgenic-knockout model wherein HGF is the transgene and the INK4 locus is knocked out, also has been found to lead to a high frequency of RMS (G. Merline, *personal communication*, 2000). Taken together, these mouse models of RMS provide evidence of the involvement of p53, pRB, and muscle differentiation pathways in the development of these tumors. Also likely is that these models will provide additional information regarding the

pathophysiology of RMS.

## PATHOLOGY

RMS falls into the broader category of small, round blue-cell tumors of childhood. The role of pathologists is to identify characteristic features, both by conventional light-microscopical techniques and by newer immunohistochemical, electron-microscopical, and molecular genetic techniques that allow a tumor to be classified as an RMS. The characteristic feature that permits such classification is the identification of skeletal myogenic lineage.

Typically, this classification consists of the light-microscopical identification of cross-striations characteristic of skeletal muscle, or characteristic rhabdomyoblasts. Immunohistochemical staining is a useful and reliable adjunctive means of identifying skeletal muscle and muscle-specific proteins or genes. These proteins include muscle-specific actin and myosin, desmin, myoglobin, Z-band protein, and MyoD.[59,60 and 61] Recently, myogenin expression was demonstrated to be present in 22 of 26 RMS specimens; however, it appeared that expression is significantly higher in ARMS than in ERMS.[62] Electron microscopy can provide additional information if light microscopy and immunohistochemistry results are ambiguous. The finding of actin-myosin bundles or Z-band material on electron-microscopical analysis provides strong support for a diagnosis of RMS.

Traditionally, RMS was classified according to the system of Horn and Enterline as embryonal, botryoid (a subtype of embryonal), alveolar, and pleomorphic.[63] The two major variants of RMS—embryonal and alveolar—have relatively characteristic histologic appearances and have specific and distinctive molecular genetic abnormalities, as described (Fig. 32-1). Histologic appearance, which is based on the identification of typical cytologic and architectural features, usually is sufficiently distinctive to permit straightforward categorization of the two subtypes. However, at times, establishing a specific diagnosis is more difficult. An international pathology study to assess agreement within and between groups of pathologists specializing in the classification of RMS highlighted the proliferation of subtle differences in diagnostic criteria that had developed since the publication of the Horn and Enterline schema.[64,65 and 66] This prompted the development of a new International Classification of Rhabdomyosarcoma schema that was demonstrated to be both highly reproducible and prognostically useful.[67] Four broad subtypes of RMS were established: (a) botryoid and spindle-cell RMS (both less common variants of ERMS), generally having a superior prognosis; (b) ERMS, generally having an intermediate prognosis; (c) alveolar (including the solid alveolar variant) RMS, generally having a poorer prognosis; and (d) undifferentiated sarcoma, also generally having a poorer prognosis. Finally, a category of sarcoma not otherwise specified was created for tumors that could not be classified into a specific subtype.

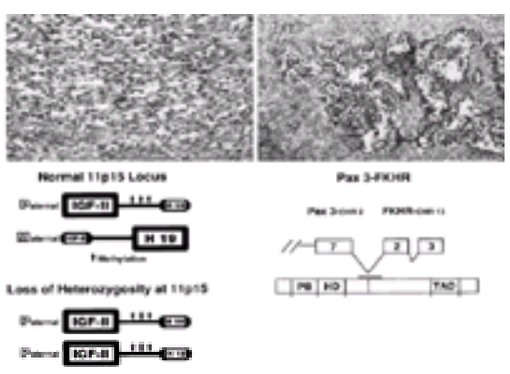


**FIGURE 32-1.** Light-microscopical appearance and genetic alterations indicative of embryonal and alveolar rhabdomyosarcoma (RMS). Embryonal RMS ( **A, C**): Typical spindle-shaped cells with stromal-rich appearance, characterized by loss of the maternal allele [loss of heterozygosity (LOH)] at 11p15 with duplication of the paternal allele. Note the paternal allele is characterized by expression of insulin-like growth factor-2 (IGF-2), whereas the maternal allele is characterized by expression of H19. Thus, LOH with paternal duplication leads to alleles with IGF-2 expression. Alveolar RMS ( **B, D**): Typical small round cells with dense appearance, lined up along spaces resembling pulmonary alveoli. These tumors are associated with a characteristic chromosomal translocation between the long arms of chromosomes 2 (more rarely, chromosome 1) and 13. This translocation fuses the paired-box (PB) and homeodomain (HD) DNA binding regions of the PAX3 gene with the transcriptional activation domain (TAD) of the FKHR gene. Horizontal line indicates fusion region of messenger RNA. (From Wexler LH, Helman LJ. Soft tissue sarcomas of childhood. In: Bast RC, Kufe DW, Pollock RE, et al. Cancer medicine, 5th edition. Hamilton: BC Decker Inc, 2000:2199.)

Under this new classification schema, embryonal tumors are diagnosed if the tumor has a stroma-rich, less dense, spindle-cell appearance and shows no evidence of an alveolar pattern. Variant forms of ERMS include the botryoid and leiomyomatous (spindle-cell) subtypes. The botryoid tumors have a particularly favorable prognosis and tend to arise almost exclusively from the bladder or vagina in infants and young children or from the nasopharynx in slightly older children.[68] Microscopically, they present as a polypoid mass growing under an epithelial surface and have as their characteristic feature the presence of a dense tumor cell layer under the epithelium (the cambium layer). The spindle-cell variants tend to arise disproportionately in the paratesticular region but may be seen also in the head and neck, extremities, and orbit.[69] These cells have a characteristic elongated, spindle appearance and grow either in a storiform pattern, with abundant collagen between the tumor cells, or in bundles, with a low to moderate amount of collagen. They almost always are associated with limited disease; they appear to have a pattern of behavior that is less aggressive than that of the classic embryonal tumors and an extremely good prognosis. Approximately two-thirds of newly diagnosed cases of RMS are of the embryonal subtype (Fig. 32-2).

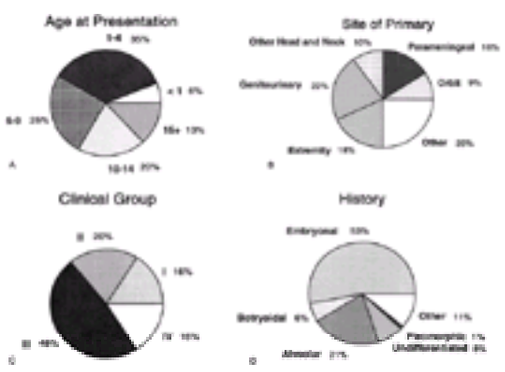


**FIGURE 32-2.** Clinical features of rhabdomyosarcoma (pooled data from Intergroup Rhabdomyosarcoma Studies I, II, and III[76,77 and 78]). **A:** Age at presentation. The median age at diagnosis is 5 years, and disease is diagnosed in almost two-thirds of patients before 10 years of age. **B:** Site of primary tumor. Approximately 35% of tumors arise in the head and neck (e.g., orbit, parameningeal sites); next most common are genitourinary tract tumors, followed by tumors of the extremities. **C:** Clinical group. Approximately half of all patients have unresectable tumors (clinical group III) at presentation. **D:** Histology. Although diagnostic criteria have evolved over time, more than 50% of all tumors are of the embryonal variety.

The presence of any alveolar pattern is sufficient to categorize the tumor as an alveolar subtype. Typically, these tumors are composed of densely packed, small, round cells lining septations that appear histologically reminiscent of pulmonary alveoli. A variant form, known as *solid ARMS*, has been identified in tumors that lack the characteristic architectural appearance (i.e., the alveolar septations) but have cells that are small, round, and densely packed.[65] The clinical behavior of the solid alveolar variant appears to be identical to that of the conventional alveolar subtype. Among newly diagnosed cases of RMS, 20% to 30% are of the alveolar subtype. Undifferentiated sarcomas generally are lacking in any defining cytologic or architectural features and fail to express antigenic markers that otherwise would allow their more precise classification. Although diagnosed largely by exclusion, these tumors generally are composed of diffuse, closely packed, large, round cells, with scanty to moderate cytoplasm and with nuclei that typically are larger than those seen in RMS. This subtype appears to have a prognosis somewhere between that of ERMS and ARMS.[70] Pleomorphic RMS is diagnosed only rarely today; if anaplastic cells are present in large aggregates or diffuse sheets, this subtype also appears

to have a poor prognosis.[71]

When the diagnosis of RMS is uncertain or in need of further support, the application of molecular diagnostic approaches is helpful. The characteristic t(2;13)(q35;q14) abnormality can be determined by reverse transcriptase–polymerase chain reaction techniques and provides definitive evidence of an ARMS. This approach often requires the availability of fresh frozen tumor tissue for RNA extraction but increasingly can be carried out on material obtained from paraffin blocks. With ERMS, the availability of highly polymorphic markers at the 11p15 locus allows for the application of polymerase chain reaction technology for rapid identification of LOH of 11p15 in paraffin sections.[72]

Evidence has been conflicting concerning the prognostic significance of histology. [65,73] Although histology was found to be an important prognostic variable in IRS-II, its significance no longer was seen with the more intensive, risk-based therapy of IRS-III (see the section Prognostic Considerations).[74,75,76,77 and 78] Previous analyses of the prognostic significance of histologic subtype in noncontemporaneously treated patients may have suffered from differences in diagnostic criteria. [68,79,80,81,82 and 83] More recent data, however, strongly support the independent prognostic significance of histology. Investigators from the National Cancer Institute and St. Jude Children's Research Hospital evaluated a group of 159 patients with RMS treated at the two institutions over a 15-year period. [65] Among patients with nonmetastatic tumors, histology was found to be an independent prognostic variable, with embryonal tumors having an outcome better than that of the identically behaving alveolar or solid alveolar tumor variants (6-year survival rate, 60% vs. 25%; $p = .001$). In a study of outcome among 264 patients with orbital RMS treated on IRS-I through IRS-III and IRS-IV pilot, the 5-year survival for the 221 patients with ERMS (and variant subtypes) was 94% versus 74% for the 24 children with ARMS ( $p<.001$).[84] Finally, a preliminary report from IRS-IV (in which, unlike that in IRS-III, treatment was identical regardless of histology) suggests that alveolar histology truly does define a population of patients' tumors that are more aggressive and have a poorer prognosis. [85]

One important caveat in the interpretation of these preliminary data is the need to perform multivariate analyses to account for the clustering of histologic subtype with site and tumor size and invasiveness. For example, a report of outcome among 139 patients, who had extremity RMS (more than two-thirds of whom had ARMS) and were treated on IRS-IV, identified group (extent of initial surgical resection) and stage (tumor size or regional lymph node positivity, or both), but not histologic subtype, as the variables most predictive of outcome.[86]

The application of molecular pathology to classification of RMS subtypes may help to resolve some of these controversies by the use of more objective criteria based on genetic differences between alveolar and embryonal tumors. It also may address questions regarding the pathogenesis of these tumors and may prove useful at defining distinct prognostic subgroups within categories of histologic subtype. For example, one recent study suggested that patients with the variant PAX7-FKHR translocation have a more favorable prognosis than do those with the more common PAX3-FKHR translocation.[87] Even more intriguing is a preliminary report suggesting a strikingly better outcome among patients with metastatic disease and variant-translocation-positive ARMS (estimated 4-year overall survival, 75% vs. 8% for patients with PAX3-FKHR-positive ARMS; $p = .0015$).[88]

## PATTERNS OF SPREAD AND CLINICAL PRESENTATION

The biologic behaviors of RMS and undifferentiated sarcoma are similar and have become increasingly well understood in the last 30 years as treatment techniques have improved. In the early prechemotherapy era, RMS had a uniformly high rate (65% to 80%) of eventual metastasis after local control measures alone. [89,90 and 91] In the current combined-modality therapy era, the development of effective adjuvant chemotherapy regimens has led to a clearer picture of the patterns of metastatic spread after treatment failure.[92,93 and 94] Fewer than 25% of patients with newly diagnosed disease have distant metastases, and almost one-half of those patients have only a single site of involvement (most commonly consisting of one or more pulmonary metastases). The lung is the most frequent site of metastasis (40% to 50%); less common sites, either isolated or in conjunction with multimetastatic disease, are bone marrow (20% to 30%), bone (10%), and, depending on the site of the primary tumor, lymph node (up to 20%).[95,96,97,98 and 99] Visceral organ metastases are rare in patients with newly diagnosed disease. These same sites are common locations for distant failure in patients who relapse after receiving systemic therapy; however, preterminally, visceral metastases (e.g., brain, liver) may be seen in up to 25% of patients.[74]

RMS and undifferentiated sarcoma produce clinically evident signs and symptoms in two main ways: the appearance of a mass lesion in a body region without history of temporally associated trauma and the disturbance of a normal body function by an otherwise unsuspected, critically located enlarging tumor (or enlarging regional or distant lymph nodes).[98,99] Typical signs, symptoms, and patterns of spread are discussed in terms of the primary tumor and are summarized here.

In the first three IRS trials, approximately 35% to 40% of all tumors arose from a site in the head or neck region (orbit, parameningeal, other head and neck); slightly fewer than 25% from the genitourinary tract (bladder and prostate, vagina and uterus, paratesticular); approximately 20% from an extremity; and the remainder from truncal primary tumors and other miscellaneous sites (approximately 10% each; Fig. 32-2).[76,77 and 78]

### Head and Neck Region

Approximately 25% of head and neck sarcomas arise in the orbit, 50% in other parameningeal sites, and 25% in nonorbital, nonparameningeal locations, such as the scalp, face, buccal mucosa, oropharynx, larynx, and neck (Fig. 32-3).[100] The gender ratio is almost equal, and the median age at diagnosis is approximately 6 years. Orbit-eyelid tumors produce proptosis and, occasionally, ophthalmoplegia. [101] Tumors in this site usually are diagnosed before distant dissemination has taken place. Regional lymph node spread is unusual, probably because the orbit is supplied only scantily with lymphatic channels. Nonorbital parameningeal sarcomas arise most commonly in the nasopharynx and paranasal sinuses, the middle ear and mastoid region, and the pterygoid-infratemporal fossae. These tumors usually produce nasal, aural, or sinus obstruction, with or without a mucopurulent and sometimes sanguinous discharge. Cranial nerve palsy, sometimes multiple, indicates direct extension toward the meninges.[102,103,104 and 105] Headache, vomiting, and systemic hypertension may result from intracranial growth of tumor after erosion of contiguous bone at the cranial base.[105,106] Autopsy studies show diffuse involvement of the cranial and spinal meninges reminiscent of central nervous system leukemia. [107] These tumors can spread also distantly, primarily to lungs or bones.[108] Craniocervical sarcomas arising in areas other than the orbit and parameningeal sites usually present as painless, progressively enlarging growths and tend to remain localized. [109,110]

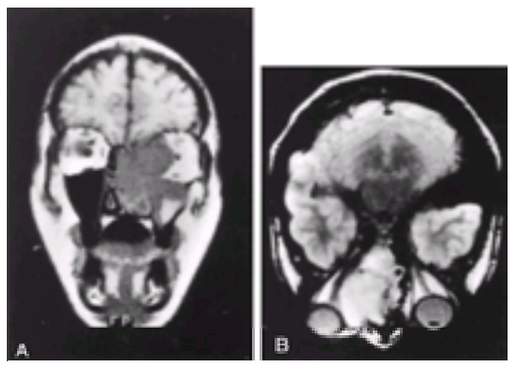


**FIGURE 32-3.** Radiographic appearance of TNM stage 3 rhabdomyosarcoma of the head and neck (parameningeal) region. **A:** Large tumor (>5-cm maximum diameter) originating in the ethmoid sinus, invading the left orbit (T2b), and eroding through the base of skull. Ipsilateral cervical lymph nodes (not shown) were clinically enlarged and pathologically involved by tumor (N1). The patient was treated for sinusitis for 1 month before the correct diagnosis was made. **B:** The tumor caused significant proptosis and nasal congestion, both of which resolved within 72 hours after the initiation of systemic chemotherapy. (From Wexler LH, Helman LJ. Pediatric soft tissue sarcomas. CA Cancer J Clin 1994;44:211, with permission.)

### Genitourinary Tract

Genitourinary tract sarcomas are seen most frequently in the bladder and prostate ( Fig. 32-4, Fig. 32-5).[111] Bladder tumors tend to grow intraluminally in or near the trigone and have a polypoid appearance on gross or endoscopic examination. Hematuria, urinary obstruction, and, occasionally, the extrusion of mucosanguineous

tissue can occur, particularly if the tumor is botryoid. Affected children usually are younger than age 4. Prostate tumors usually produce large pelvic masses with or without urethral strangury; constipation may occur. These tumors can occur in infants or older children; even adults may be affected. [112] Bladder tumors tend to remain localized, but prostate tumors often disseminate early to lungs and sometimes to bone marrow or bones. [113,114]

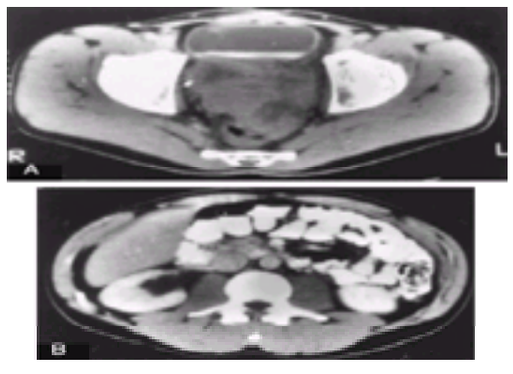

**FIGURE 32-4.** Radiographic appearance of rhabdomyosarcoma of the bladder-prostate region. **A:** This patient presented with a large infiltrating, unresectable tumor (T2b, TNM stage 3, clinical group III) causing obstipation and a palpable mass on rectal examination. **B:** These tumors can grow very large before they are detected, often leading to obstruction of the ureters and hydronephrosis (seen in the right kidney). Cystectomy or pelvic exenteration rarely is indicated in the primary surgical management of these tumors. (From Wexler LH, Helman LJ. Pediatric soft tissue sarcomas. CA Cancer J Clin 1994;44:211, with permission.)

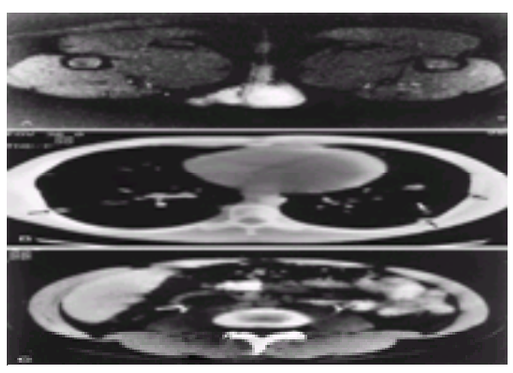

**FIGURE 32-5.** Radiographic appearance of rhabdomyosarcoma of the paratesticular region. **A:** This patient presented with a firm, nontender, small scrotal mass (T1a) that was completely resected (clinical group I). He subsequently was found to have distant pulmonary **(B)** and lymph node **(C)** metastases (*black and white arrows*) and multiple bony metastases (not shown), which increased the stage of the lesion to TNM stage 4.

Male and female genital tracts can harbor sarcoma. [115] Vaginal tumors commonly are botryoid and are found almost exclusively in very young children who may have a mucosanguineous discharge reminiscent of that seen with a foreign body. [116] Cervical and uterine sarcomas are diagnosed more commonly in older girls than in infants and present with a mass, with or without vaginal discharge. Regional nodal involvement is uncommon. [98,99] Paratesticular tumors usually produce painless, unilateral scrotal or inguinal enlargement in prepubertal or postpubertal male patients. The risk of tumor dissemination to regional retroperitoneal lymph nodes appears to be linked closely to age at diagnosis, being distinctly uncommon in boys younger than age 10 and being present in 50% or more of older boys. [117] Alveolar histology is distinctly unusual in sarcomas of the genitourinary tract. [111]

### Extremities

Sarcomas of the extremity are characterized by swelling in the affected body part (Fig. 32-6). The male-female ratio is approximately 1:1. Pain, tenderness, and redness may occur. Between one-half and three-fourths of these tumors are alveolar. [86,118] Regional lymph node spread may be found in up to one-half of patients undergoing surgical exploration and is more likely if the primary tumor is an ARMS rather than an ERMS or undifferentiated sarcoma. [86,92,118,119 and 120] The tumors can be extensive because of their propensity to spread along fascial planes. The fact that injuries are frequent and expected on the extremities of school-aged children may lead to a delay in diagnosis.

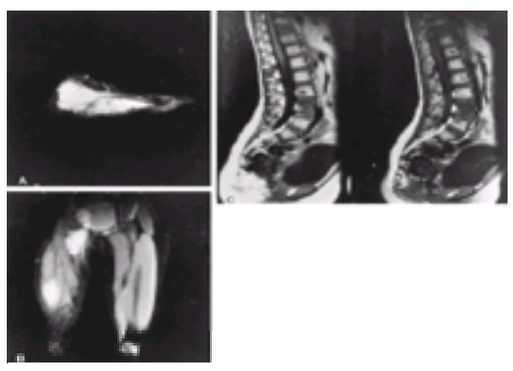

**FIGURE 32-6.** Radiographic appearance of rhabdomyosarcoma of the extremity. This patient presented with a painless, rapidly enlarging mass of the plantar surface of the right foot **(A)**. Biopsy of the mass revealed it to be an alveolar rhabdomyosarcoma. These tumors typically develop in adolescents and are associated with an aggressive clinical course and a propensity to spread to lymph nodes. Physical examination and radiographic staging studies demonstrated the presence of soft tissue and lymph node metastases in the popliteal fossa (not shown) and in the distal anterior thigh and inguinal pelvic region **(B)**, as well as osseous metastases involving numerous vertebral bodies **(C)**. (From Wexler LH, Helman LJ. Pediatric soft tissue sarcomas. CA Cancer J Clin 1994;44:211, with permission.)

### Trunk

Truncal sarcomas are similar in evolution to those of the extremities in that they exhibit all histologic types and have a tendency for local recurrence despite wide local excision and for distant spread. They are of relatively large diameter as compared with tumors of the head and neck or of the bladder. [121,122] Contiguous involvement of the thoracolumbar spine may exist, depending on the location of the primary lesion, but regional lymph node spread is unusual.

### Other Sites

#### *Intrathoracic and Retroperitoneal and Pelvic Regions*

Intrathoracic and retroperitoneal-pelvic tumors can become large before a diagnosis is made because they are deep within the body. [123] They often are incompletely accessible to a surgeon, because vital vessels usually are surrounded and wide infiltration is the rule; however, more recent data fail to support the notion that

differences in outcome for patients with thoracic tumors are accounted for by a higher proportion of patients with unresectable disease. [124] Patients with tumors in these locations have a higher-than-expected risk of local recurrence despite combined-modality treatment. Aggressive attempts at initial or delayed surgical resection, combined with appropriate postoperative radiotherapy (RT), may improve prognosis. [125]

### *Perineal and Perianal Region*

Lesions in the perineal-perianal region are unusual. They can mimic abscesses or polyps and often are alveolar. [126] In the IRS, a relatively high incidence of regional lymph node involvement was reported for the first series of patients with disease located in this region. [127]

### *Biliary Tract*

Biliary tract tumors are even rarer than perineal-perianal tumors. They often produce obstructive jaundice, spread within the liver, and then spread to the retroperitoneum or lungs.[128,129 and 130] Aggressive surgical resection appears to be less important to good outcome for tumors in this location. [131]

### *Miscellaneous Potential Sites of Spread*

Occasionally, the liver, brain, trachea, heart, breast, or ovary may harbor a primary sarcoma. [132,133,134,135,136 and 137] In some cases, no definite primary site can be determined.[138]

## METHODS OF DIAGNOSIS

The differential diagnosis of RMS and undifferentiated sarcoma includes other oncologic entities and an assortment of nononcologic conditions. Trauma may produce an enlarging soft tissue mass, especially over the extremities, face, or trunk. Usually, a history of an accident is available, and an associated hematoma is tender and discolored. Sarcomas usually are nontender and impart no unusual hue to the overlying skin or subcutaneous tissue. Growth of a nontender mass, especially without a clear-cut history of trauma, always should alert the examiner to consider biopsy, especially if expansion is confirmed by repeated observations over 1 to 2 weeks. A mass within a body cavity can produce obstruction or discharge; both mandate a biopsy.

On rare occasions, cystitis may produce imaging and cystoscopic findings that mimic the appearance of RMS of the bladder; however, follow-up imaging almost always shows a return to normal over the course of 1 to 2 weeks, precluding the need for biopsy under those circumstances. [139] Occasionally, a histologically benign lesion, such as a lipoma, rhabdomyoma, or neurofibroma, may be diagnosed; if so, complete surgical removal should be performed if mutilation can be avoided. Rarely, an unusual condition, such as myositis ossificans, pyogenic myositis, or inflammatory myofibrohistiocytic proliferation (also known as *pseudosarcomatous myofibroblastic tumor* or *inflammatory pseudotumor of the bladder*), may be discovered.[140,141,142,143 and 144] This last condition is a recently described, rare, benign lesion that may create difficulty in differentiation from RMS by conventional diagnostic techniques. [145] It may present as an ulcerated, hemorrhagic, polypoid growth with intraluminal invasion that is found in the course of routine radiologic evaluation of hematuria and dysuria (not uniformly associated with documented cystitis).

Biopsy should be considered also if a young person has a mass and is failing to thrive, even if the affected region is tender and the patient is febrile (if appropriate studies for infection have been nonproductive), because a treatable neoplasm may be the underlying disorder. Other childhood malignancies can mimic RMS or undifferentiated sarcoma. Non-Hodgkin's lymphoma, neuroblastoma, and Ewing's sarcoma can simulate sarcoma at the light-microscopical level, and special stains, electron-microscopical ultrastructure studies, monoclonal antibody assays, and collection of urine for catecholamine excretion studies may be necessary to differentiate these entities. Occasionally, a leukemic chloroma or collection of histiocytes (e.g., Langerhans' cell histiocytosis) can produce unilateral proptosis or a mass in another body region, which should undergo biopsy to establish the correct diagnosis. [146,147]

After the diagnosis of RMS or undifferentiated sarcoma has been entertained and even without confirmatory pathologic material, several clinical and radiographic studies may be in order to define the limits of the lesion and to seek evidence of spread. A complete physical examination should be performed, with particular attention paid to regional lymphatic structures and to the surrounding tissues. Laboratory studies that should be obtained simultaneously include a complete blood count with differential count, serum electrolytes, blood urea nitrogen and creatinine levels, liver function tests, serum calcium, phosphorus, and magnesium levels, and a uric acid level, in anticipation of chemotherapy. Patients with bone marrow metastases from a primary sarcoma may have altered peripheral blood values, but bilateral bone marrow aspirations and core needle biopsies should be performed routinely even in the absence of altered blood counts or obvious metastases. Although disseminated intravascular coagulation is uncommon, even among patients with bone marrow involvement, baseline coagulation studies (prothrombin time, activated partial thromboplastin time, fibrinogen) should be performed in all patients, and appropriate supportive care measures should be initiated if evidence of the condition is found. [111] Metastatic bone involvement rarely can be complicated by hypercalcemia. [148,149]

Radiographic studies should include plain films of the affected part and a skeletal survey. Nuclear medicine scans using technetium-99m diphosphonate may be useful in the search for osseous metastases. [150] Technetium-99m bone scans are highly sensitive and relatively specific for detecting osseous metastases and probably are more reliable than a routine skeletal survey. [151,152] Gallium-67 can be concentrated in the bowel and in areas of inflammation, and it is not usually a routine part of the diagnostic workup. [152]

Computed tomography scans, with or without contrast enhancement, long have been the standard imaging modality. Preoperative scanning is critical to enable the radiation therapist to assess the volume at risk for subclinical tumor invasion and to plan treatment fields. [153] Imaging of the abdomen and pelvis also may be useful for detecting clinically occult abnormalities of the genitourinary tract. Ultrasonographic examinations may be especially useful as an adjunct to computed tomography in serial assessment of tumors of the pelvis (including the bladder, prostate, and retroperitoneum), because the characteristic water density of the urine-filled bladder helps in localization. [154] Ultrasonography does not use radiation, and dye injection is unnecessary. Magnetic resonance imaging is increasingly becoming the imaging modality of choice, especially for head and neck, extremity, and pelvic tumors, because of its multiplanar capability, its ability to attenuate bone artifact, and the superior soft tissue contrast that it provides. [155,156,157 and 158]

### Staging

Assessing the extent of the tumor in every patient is critical, because therapy and prognosis depend on the degree to which the mass has spread beyond the primary site. Patients with localized, surgically removable tumors have a prognosis better than that in those whose disease has produced clinically detectable metastatic deposits. Two major staging systems currently are employed in combination: the older Children's Oncology Group surgicopathologic staging system (designated by the phrase *clinical group*, or *CG*, followed by a number), developed by the IRSG in 1972 (Table 32-1), and the more recent pretreatment, site-modified TNM staging system (indicated simply by the word *stage* followed by a number), developed by the IRSG for use in IRS-IV (Table 32-2).[159] The CG system defines patients by the extent of their initial surgery, further subclassifying patients with microscopic residual disease (CG II) with or without regional nodal involvement (IIB, C, or IIA, respectively). The TNM system, which was evaluated retrospectively by numerous investigators and was shown to be highly predictive of outcome, divides patients into favorable and unfavorable sites and requires up-staging of patients with unfavorable-site tumors that are large (more than 5 cm) or have clinical evidence of regional nodal involvement (N1).[81,82 and 83,159] Favorable sites include the orbit and eyelid and other nonparameningeal head and neck structures, as well as nonbladder, nonprostate genitourinary locations (paratesticular and vulva, vagina, or uterus). Among the unfavorable sites are the extremities (including the buttocks and perineum), urinary bladder and prostate, cranial parameningeal sites, and the trunk and retroperitoneum.

| Clinical group | Extent of disease and surgical result |
|---|---|
| I | A. Localized tumor, confined to site of origin, completely resected<br>B. Localized tumor, infiltrating beyond site of origin, completely resected |
| II | A. Localized tumor, gross total resection, but with microscopical residual disease<br>B. Locally "extensive" tumor (spread to regional lymph nodes), completely resected<br>C. "Extensive" tumor (spread to regional lymph nodes), gross total resection, but with microscopical residual disease |
| III | A. Localized or locally extensive tumor, gross residual disease after biopsy only<br>B. Localized or locally extensive tumor, gross residual disease after "major" resection (>50% debulking) |
| IV | Any size primary tumor, with or without regional lymph node involvement, with distant metastases, irrespective of surgical approach to primary tumor |

**TABLE 32-1. CLINICAL GROUP STAGING SYSTEM EMPLOYED IN INTERGROUP RHABDOMYOSARCOMA STUDIES I THROUGH III**

| Stage | Sites | T Invasiveness | T size | N | M |
|---|---|---|---|---|---|
| 1 | Orbit<br>Head and neck[b]<br>Genitourinary[c] | T1 or T2 | a or b | N0, N1, or Nx | M0 |
| 2 | Bladder/prostate<br>Extremity<br>Cranial parameningeal<br>Other[d] | T1 or T2 | a | N0 or Nx | M0 |
| | | T1 or T2 | b | N0, N1, or Nx | M0 |
| 4 | All | T1 or T2 | a or b | N0 or N1 | M1 |

[a]T (tumor): T1, confined to anatomic site of origin; T2, extension; a, ≤5 cm in diameter; b, >5 cm in diameter. N (regional nodes): N0, not clinically involved; N1, clinically involved; Nx, clinical status unknown. M (metastases): M0, no distant metastases; M1, distant metastasis present.
[b]Excluding parameningeal.
[c]Nonbladder/nonprostate.
[d]Includes trunk, retroperitoneum, and so on.

**TABLE 32-2. TNM STAGING[a] OF RHABDOMYOSARCOMA: TNM PRETREATMENT STAGING CLASSIFICATION FOR INTERGROUP RHABDOMYOSARCOMA STUDY IV**

The likelihood of infiltration of regional lymph nodes or adjacent structures varies with the site of the primary tumor, ranging from as low as 5% for head and neck tumors to as high as 50% for extremity and paratesticular tumors (in older boys).[86,98,99] Imaging studies and physical examination findings usually are adequate for establishing the presence of regional nodal involvement. Any palpably or radiographically enlarged regional lymph node at any site should be removed and submitted for pathologic examination. Routine surgical sampling of radiographically “benign” regional nodes is unwarranted with two important exceptions: All cases of extremity RMS should undergo aggressive sampling of regional nodal basins, and older boys (age 10) with paratesticular tumors should undergo ipsilateral lymph node dissection. RT is delivered to the region if tumor involvement of nodes is found on pathologic examination. Radiographic studies and bone marrow examination are used to ascertain whether distant metastases are present; histologic verification of radiographic abnormalities is not required. However, where treatment issues will be determined by the presence or absence of metastatic disease, surgical evaluation of an equivocal radiographic abnormality may be warranted. Histologic subtype does not affect either the stage or clinical group, although it may have an impact on systemic or local treatment choices [e.g., the use of local irradiation in completely resected (group I), low-stage (1 to 2) ARMS].[160]

### Prognostic Considerations

The identification of prognostic variables is of major importance in understanding the behavior of sarcomas and developing careful clinical trials, the goals of which are to improve survival for all patients with RMS and undifferentiated sarcoma and to reduce morbidity. Several key prognostic variables have been identified and currently are being used in IRS-V to define risk-adapted therapy ( Table 32-3). These variables, which define distinct groups of patients with excellent, very good, intermediate, and poor prognoses, include (a) the presence or absence of distant metastases (with two important exceptions, discussed later); (b) site (favorable vs. unfavorable, with the most favorable site being the orbit); (c) surgical resectability (groups I and II vs. group III, excluding the orbit); (d) histology (ERMS and variants vs. ARMS and undifferentiated sarcoma); and (e) age.[161] As discussed, recent data suggest that the specific type of molecular abnormality present in ARMS may be prognostically significant, particularly for patients with metastases.

**TABLE 32-3. PROGNOSTIC STRATIFICATION FOR RHABDOMYOSARCOMA**

Patients with no detectable metastases at diagnosis fare much better than do those with widespread disease ( Fig. 32-7).[76,77] and [78,162] Among patients with localized sarcoma, those with completely excised tumors (CG I) have a survival rate better than that of those with microscopic residual tumor or with excised but regionally extensive lesions (CG II). Among patients with microscopic residual disease, those without regional nodal involvement (CG IIA) or with completely resected regional nodes (CG IIB) fare better than do those with both [CG IIC; 5-year failure-free survival (FFS), 75% vs. 74% vs. 58% for patients with CG IIA, IIB, and IIC, respectively; $p = .004$].[163] Patients with gross residual disease (CG III) do not fare as well; however, for patients with ERMS, outcome within this group varies according to whether the tumor originates in a favorable site (92% 3-year FFS) or an unfavorable site (75% 3-year FFS). [164] Although young patients (younger than age 10) with metastatic ERMS[165] and those with variant-translocation-positive (PAX7-FKHR) metastatic ARMS[88] may have an intermediate prognosis (approximately 50%), the prognosis for most patients with metastatic RMS remains grim. The most meaningful prognostic variable is the response to treatment, because those in whom complete obliteration of the tumor never is achieved (i.e., a complete pathologic response to therapy) do not survive. Early response to treatment appears to correlate with a better outcome.[166]

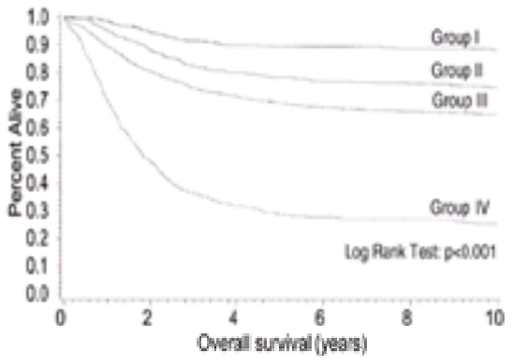


**FIGURE 32-7.** Survival of patients treated on Intergroup Rhabdomyosarcoma Studies (IRS)-I, -II, -III/IV-P (IV pilot), and -IV by clinical group at diagnosis. A significant difference is seen in outcome by extent of initial surgical resection, with the best outcome among patients with completely resected tumors (group 1), followed by those with microscopical residual (group 2) and gross residual (group 3) disease. Patients with metastatic disease (group 4) at diagnosis fare poorly. (JR Anderson, *personal communication,* 2000.)

## TREATMENT

The three currently recognized modalities of treating children with sarcomas are surgical removal (if feasible), RT for control of residual bulk or microscopic tumor, and systemic chemotherapy for primary cytoreduction and eradication of gross metastases and micrometastases. Much of the information regarding current use of these modalities derives from therapeutic programs developed by the IRSG.

### Principles of Surgical Management

Surgery is the most rapid way to ablate the disease, and it always should be used if subsequent function or cosmesis will not be greatly impaired. In some sites, such as the vagina and female genital tract, the urinary bladder, the orbit, and the biliary tract, aggressive surgical treatment is unwarranted. In other sites, such as the head and neck, a diagnostic incisional biopsy may be the only feasible surgical procedure because of proximity to vital blood vessels and nerves, cosmetic considerations, or both. If microscopic residual disease is found after an initial excision or if the initial operation was carried out without knowledge of the type of neoplasm involved, reexcision of the area may be indicated. In localized lesions of the trunk and extremities, improvement in survival time can be produced by primary surgical reexcision of all residual tumor before the initiation of chemotherapy. [124,167] Occasionally, debulking surgery is used to reduce the volume of residual tumor beyond that which would remain after incisional biopsy alone. Carefully reviewed data in support of this theoretically reasonable maneuver for children with RMS or undifferentiated sarcoma are not available.

Second-look surgical procedures have been evaluated in three clinical circumstances: (a) to verify pathologically the completeness of an apparently complete clinical (radiographic) remission for the purpose of then eliminating further local control measures, such as RT; (b) to resect any residual viable tumor cells that have survived after induction chemotherapy and local irradiation; and (c) to permit a reduction in the dose of radiation in patients who initially present with group III tumors.

The International Society of Pediatric Oncology enrolled 425 patients in their 1984 Malignant Mesenchymal Tumors Study, which consisted of nonradical surgery or biopsy followed by three to six cycles of induction chemotherapy with vincristine, actinomycin-D, and ifosfamide (VAI). [168] Definitive local treatment (surgery or RT) depended on the response to chemotherapy. Additional local therapy was not given to patients having no evidence of residual tumor after induction therapy. Of 237 patients with initially incompletely resected, nonparameningeal localized tumors, 140 (including 92 with RMS) achieved a complete clinical response to induction chemotherapy. Approximately one-half of these patients, who received no further local therapy, ultimately had local recurrence, and no difference was noted in the local recurrence rate between patients undergoing biopsy confirmation of complete remission status (26 of 52) and those followed up clinically (18 of 39). Therefore, as a strategy to permit the withholding of definitive local therapy after the achievement of a complete clinical response, biopsy confirmation is inappropriate because of the high rate of false-negative results and the unacceptable local relapse rate when such an approach is followed.

Data regarding the role of secondary (second-look) operations to resect residual viable tumor after the administration of definitive local therapy were collected in CG III patients enrolled in IRS-III, for whom a delayed resection of the residual primary tumor was recommended, whenever possible, after the first 20 weeks of treatment (i.e., after the completion of induction chemotherapy and local RT). Second-look operations were found to produce complete responses by removing residual tumor after primary chemotherapy and RT and to improve the accuracy of clinical and radiologic assessment of response by providing tissue for pathologic examination.[78,169,170] Sixty-four percent of CG III patients who underwent secondary operations in radiographic partial remission were found to be in complete remission and, more important, 52% of those who underwent secondary operations after achieving only a minor response (less than 50% regression in cross-sectional tumor diameter) were converted to complete remission status by the procedure. These findings formed the basis of the IRS committee's cautious endorsement of the role of second-look surgery for CG III patients in partial remission after induction chemotherapy and RT. The committee acknowledged, however, that the contribution of second-look surgery to the improvement in long-term survival of these patients could not be assessed adequately because of the frequent concomitant use of alternative induction therapy in those same persons. [78]

Finally, investigators at St. Jude Children's Research Hospital reported maintenance of local control in 22 of 28 patients with initial group III tumors treated with lower-than-standard dose irradiation after being rendered free of gross disease with chemotherapy alone (n = 16) or in conjunction with surgery (n = 12). [171] A trend occurred toward improved local control within this group among patients receiving 40 Gy versus those receiving less than 40 Gy (15 of 17 vs. 7 of 11; $p<.14$). These preliminary results form the basis for a more formal evaluation of the role of second-look surgery in reducing the risk of local recurrence (and the dose of local irradiation) in patients with initially unresectable tumors being treated on IRS-V.

#### *Tumors of the Head and Neck*

Head and neck tumors, with the exception of those arising in relatively superficial locations, rarely are amenable to wide local excision. Incisional biopsy for diagnostic purposes usually is all that is feasible and, in the case of orbital tumors, it is all that is necessary, given the excellent results achieved with chemotherapy and RT regimens. Unless clinically suspicious nodes are present, routine cervical lymph node sampling is unnecessary, because the incidence of regional lymph node involvement is fairly low. The availability of highly skilled otolaryngology and craniofacial reconstruction teams at select institutions may permit the resection of some tumors that otherwise would be unresectable.[172]

#### *Tumors of the Genitourinary Tract*

### Paratesticular Tumors

Paratesticular tumors should be removed by radical inguinal orchidectomy with resection of the entire spermatic cord. An inguinal approach is used to avoid scrotal contamination, which is likely if a transscrotal biopsy is performed. The necessity of subsequent retroperitoneal lymph node dissection (RPLND), which is undertaken to determine whether regional retroperitoneal lymph nodes harbor tumor deposits, has been controversial. Although at least one European study had advised avoidance of RPLND if radical inguinal orchiectomy resulted in complete microscopic excision and if radiographic imaging studies were normal, [173] the IRS committee continued to recommend the procedure in IRS-III. RPLND was performed in 121 patients with nonmetastatic paratesticular RMS treated on IRS-III. [174] Only 14% of patients without radiographic evidence of lymph node involvement were found to have pathologically involved nodes, whereas 94% of those with radiographically enlarged nodes were confirmed to have nodal involvement. Only patients with pathologically confirmed positive nodes received RT in addition to postoperative adjuvant chemotherapy. The 5-year survival rate was significantly better for those patients with clinically negative lymph nodes than for those with clinically positive nodes (96% vs. 69%; $p<.001$); however, treatment failures were usually caused by distant, not locoregional, lymph node disease recurrence. Therefore, routine RPLND was not recommended in the IRS-IV trial for patients with completely resected localized tumors and negative imaging studies, although systematic retroperitoneal lymph node sampling *was* recommended, including ipsilateral high and low infrarenal (caval, interaortocaval, and aortic) and bilateral iliac nodes. A preliminary analysis of IRS-IV data suggested that this approach resulted in a dramatic down-staging of patients (from group II to group I) and was associated with a

worse outcome, particularly for boys age 10 or older who were treated with two-drug (VA) chemotherapy.[175] These findings form the basis of the current IRS recommendation to perform ipsilateral RPLND in all boys who are at least 10 years of age at diagnosis. Surgical resection of enlarged lymph nodes in younger boys also is warranted to down-stage them from group III to group II. Given the inferior outcome of older boys treated on IRS-III (wherein surgical exploration was required),[174] an alternative approach to ipsilateral RPLND in such patients is the routine administration of more intensive three-drug chemotherapy (VA plus cyclophosphamide or ifosfamide).[176] Similarly, the necessity of postoperative irradiation in such patients with intensively treated resected retroperitoneal nodes has been called into question.[176] The small number of patients with known nodal tumor renders execution of a controlled study difficult.[177,178]

**Vulvar, Vaginal, and Uterine Tumors**

Wide local excision of vulvar and vaginal tumors rarely is indicated before the commencement of primary chemotherapy. These tumors usually respond sufficiently well to induction chemotherapy to render them easily resectable, often with histologically negative margins. Tumors of the proximal vagina may require hysterectomy with partial or complete vaginectomy. Uterine tumors usually are managed without oophorectomy in the absence of overt ovarian involvement. Most patients are not managed initially with hysterectomy but, for those in whom hysterectomy is performed, distal vaginal preservation usually is possible. Second-look surgery and radical resection of lesions in these areas usually are reserved for patients who have gross residual disease after the initial surgical resection and have either failed to achieve a complete radiographic response within 6 months after the completion of induction chemotherapy and RT or have had early disease progression after the commencement of chemotherapy and RT. Two recent reports have further clarified the role of conservative surgery in the management of these tumors.[179,180]

**Bladder and Prostate Tumors**

Management of tumors arising in these locations has evolved from a primary surgical approach (pelvic exenteration and total cystectomy) to a multimodal approach at present.[181,182,183] and [184] Radical surgical methods resulted in excellent rates of local control, but the morbidity of these operations was considered unacceptable. Current guidelines recommend complete resection for only those patients in whom preservation of bladder and urethral function can be assured.[185,186] Partial cystectomy, which usually is reserved for tumors arising in the dome of the bladder, can be performed either before the onset of chemotherapy and RT or after induction chemotherapy with or without RT. This approach results in no compromise in the survival rate but a comparable or perhaps even higher rate of preservation of bladder function than with other treatment modalities.[181,182] Total cystectomy and anterior pelvic exenteration are reserved for patients who do not achieve local control with the combination of chemotherapy and RT and have been reported to result in survival rates higher than 80% if they are performed in the absence of distant dissemination.[183,184]

***Tumors of the Extremities***

Initial complete surgical removal of extremity sarcomas should be attempted, provided that limb function will not be greatly impaired, because the prognosis is considerably worse if grossly visible tumor is left behind.[167,168] Because up to one-half of patients with extremity tumors have regional lymph node involvement, [86] sampling of clinically negative regional lymph nodes was recommended in IRS-IV and is required in IRS-V. Biopsy of clinically suspicious lymph nodes should commence with the most proximal nodes before proceeding to dissection or aggressive nodal sampling. Involvement of the ipsilateral supraclavicular lymph nodes for upper extremity tumors and iliac or para-aortic lymph nodes, or both, for lower extremity tumors is considered evidence of distant spread (stage 4). Amputation usually is not necessary, although it may be considered for patients with extensive lesions involving the bone or major neurovascular structures and for patients for whom RT probably will result in significant impairment of limb function.

***Tumors Arising in Other Sites***

Surgical removal should be attempted for truncal lesions.[188] Tumors arising in the pelvis, retroperitoneum, or intrathoracic area often cannot be removed completely because of infiltration or encirclement of major blood vessels or nerves or because of a surgeon's unwillingness to perform exenteration for pelvic-retroperitoneal tumors. Wide local resection of chest wall tumors consists of removal of the entire soft tissue mass and a bloc of uninvolved tissue extending at least one rib above and below the lesion.[125] An analysis of outcome among 84 IRS-II and IRS-III patients with thoracic sarcomas demonstrated inferior outcome among group I patients (7 of 13 suffered local recurrence), suggesting that microscopic residual disease is present in some patients and supporting the more routine application of primary reexcision, particularly in the presence of a question about the adequacy of margins.[124]

***Surgical Management of Metastatic Disease***

The role of surgical metastectomy in improving the outcome of patients with distant tumor spread is unclear. In a retrospective study of outcome after resection of pulmonary metastases in 152 patients with childhood sarcomas (Ewing's sarcoma, osteosarcoma, RMS, and other high-grade NRSTS) treated at the National Cancer Institute, Temeck et al.[189] found a uniformly poor outcome among patients with RMS. Nonetheless, given the poor outcome with conventional chemotherapy alone for such patients, removal of metastatic deposits (e.g., pulmonary nodules) may be beneficial in selected patients who otherwise have responded well to induction chemotherapy, particularly if potentially active chemotherapeutic agents or RT approaches still are available.

***Complications of Surgery***

Complications of operative management are related to tumor sites. Any procedure involving the skin and subcutaneous tissue produces a scar and loss of tissue in the region from which bulk tumor is removed. A surgeon's experience is critical in executing the proper operation. Radical regional lymph node dissections are discouraged because of subsequent scarring and lymphedema and because no convincing data substantiate that radical node dissection is therapeutic in treating pediatric RMS or undifferentiated sarcoma. Skill is especially important in the surgical exploration of lesions arising in the head and neck, where major blood vessels and important nerves are so closely apposed. In the genitourinary region, total cystectomy for bladder or prostate tumors currently is deferred until viable malignant cells clearly have persisted despite chemotherapy and RT. In patients with paratesticular sarcoma, bilateral RPLND can produce retrograde ejaculation and therefore is discouraged.

**Principles of Radiotherapy**

RT is a major tool in the treatment of children with RMS and undifferentiated sarcoma. RT can eradicate residual tumor cells from sites at which surgical therapy alone cannot ablate the mass, especially in the head, neck, and pelvis. Soft tissue sarcomas infiltrate so widely that after simple excision or enucleation, without wide excision, RT, or chemotherapy, local recurrence rates approximate 75%.[190] Soft tissue sarcomas were considered insensitive to RT before 1960, when Dritschilo et al.[191] first reported a local control rate of 96% for 27 children who were younger than age 16, had RMS or undifferentiated sarcoma, and received 5,500 to 6,500 cGy, delivered by a 4-mV or 8-mV accelerator.

Current RT guidelines have evolved over time from the logical and stepwise approach of the sequential Intergroup studies.[192,193,194,195,196,197,198,199] and [200] Daily fractions of between 180 and 200 cGy are standard; smaller daily fractions of 150 cGy may be used when large fields (e.g., entire abdomen) must be treated. A cumulative dose of between 41.4 and 45.0 Gy generally is sufficient to control microscopic residual disease, whereas higher cumulative RT doses of between 50.4 to 54.0 Gy are needed to control gross residual disease. It is important that accurate pretreatment images be obtained and that the treatment field encompass both the initial pretreatment tumor volume and a margin (usually 2 cm) of normal surrounding tissue. A “cone-down” or “shrinking-field” technique may be used at doses in excess of 36 to 41 Gy for patients whose initially unresectable tumors have responded to neoadjuvant therapy. With the exception of patients who have invasive parameningeal tumors (base-of-skull erosion, intracranial extension, cranial neuropathies), for whom treatment usually is commenced within the first several days to weeks, most patients can have treatment delayed safely until 9 to 12 weeks after diagnosis, after a period of neoadjuvant chemotherapy, during which tumor regression is the norm. Delaying the initiation of RT beyond 18 weeks may be associated with a reduced likelihood of achieving local control and should be avoided except in the rarest of circumstances. In the absence of overt meningeal involvement, whole-brain irradiation is unwarranted for cranial parameningeal tumors.

RT guidelines have been much simplified in the current IRS-V, with an underlying strategy of reducing the dose of radiation for most patients. This is being accomplished primarily in conjunction with delayed surgical resection for patients with alveolar tumors and those with initially unresectable embryonal tumors at unfavorable sites. In other locations, such as the orbit, a radiation dose reduction to 45 cGy is being evaluated prospectively in an effort to reduce late effects.

The role of RT in the local management of patients with initially completely resected tumors (CG I) recently was elucidated in a report from the IRSG looking at

outcome in 439 patients treated in IRS-I to IRS-III.[160] Pretreatment factors that were identified as being associated with inferior outcome were tumor size greater than 5 cm, sites other than the genitourinary tract, and alveolar or undifferentiated histology. ARMS patients who received RT had a significantly improved outcome as compared to those who did not. Current IRS-V treatment guidelines, therefore, recommend that virtually all patients with completely resected ARMS and undifferentiated sarcomas receive postoperative RT to a dose of at least 36 Gy. An analysis of local treatment failure among patients with CG III tumors treated in IRS-II reported an overall local control rate of 78%; a subset of patients with bulky tumors (greater than 10 cm) or tumors originating in unfavorable sites (chest, pelvis, extremity, trunk) were identified as being at especially high risk of local treatment failure. [199]

One of the major therapeutic objectives of IRS-IV was to evaluate the effect on local control of conventional fractionation RT (using daily fractions of 180 cGy to a cumulative dose of 50.4 Gy) versus hyperfractionated RT (HFRT; using twice-daily fractions of 110 cGy, separated by at least 6 hours, to a cumulative dose of 59.4 Gy) for patients with group III tumors.[200] This approach was piloted by investigators at St. Jude Children's Research Hospital, who demonstrated an absolute 2-year continuous local tumor control rate of 75% with minimal late radiation morbidity in 14 patients with CG III and CG IV tumors and persistent gross residual disease after induction chemotherapy.[201] A pilot study (IRS-IV-P) of HFRT confirmed the feasibility of administering concurrent HFRT and intensive chemotherapy and suggested that the acute toxicities of hyperfractionation were less severe than those seen with conventional fractionation RT protocols. [200] A formal analysis of local disease control in IRS-IV by type of RT administered has not yet been published. It is, however, possible to infer from the results of other recently published reports that HFRT is unlikely to have resulted in a significant improvement in local disease control. [86,164]

Sequelae of treatment are numerous. RT can produce an acute reaction characterized by erythema and swelling of the irradiated volume, which can lead to desquamation if extreme. The later effects of radiation are loss of function or growth, chiefly because of fibrosis, which increases with increasing dose and volume and diminishes with increasing age of the patient. [202,203] Fully 70% of patients with orbital RMS have impaired vision; almost one-half of patients with nonorbital RMS fail to maintain their initial height velocity, and treatment with supplemental growth hormone is not uncommon. [204,205,206] and [207]

Other controversies in the area of RT for the local control of RMS include the role of primary RT for patients with orbital tumors, [208,209] the problem of poor local control (and overall survival) in patients with cranial parameningeal tumors and extensive bony erosion, [106,210] the issue of the optimal timing of local irradiation relative to the initiation of postoperative systemic chemotherapy,[211] and the related questions of what constitutes the minimally acceptable dose of RT that can be administered without compromising local control and under what circumstances such reduced-dose radiation can be administered safely. [212,213] and [214] Although differences in practice continue within the international community, data are convincing that local RT improves outcome for patients with parameningeal RMS. [215] The role of whole-lung RT (generally to a cumulative dose of 1,440 cGy) for patients who present with overt pulmonary metastases is unclear; however, some treatment protocols continue to recommend it—a reasonable practice if a state of minimal residual disease can be achieved—given the radiosensitivity of RMS.

Techniques other than traditional external-beam megavoltage RT sometimes may be considered. One technique employs radiation implants, especially for children with small, critically located tumors of the head and neck, bladder, prostate, vagina, or extremity. [216,217,218] and [219] Because the dose is delivered to a carefully restricted volume, adjacent normal structures receive less scattered radiation and may be expected to have less fibrosis. Another newer approach for large, deeply seated tumors is treatment of the tumor by a radiotherapist under direct vision while it is exposed in the operating room. More data regarding pediatric patients are needed to assess the utility of this method.[220]

Finally, advances in the ability to incorporate three-dimensional imaging into radiation treatment planning have led to exploration of the use of three-dimensional conformal RT and intensity-modulated RT as particularly promising approaches to maximize the dose of radiation delivered to tumor-bearing tissue while minimizing the dose received by normal surrounding structures. [221,222] and [223] At present, only limited data address the use of these techniques in treating RMS. [224,225] and [226] Still newer biologically functional imaging techniques, such as positron emission tomography and nuclear magnetic resonance imaging and spectroscopy may be combined with the “dose-sculpting” ability of intensity-modulated RT to generate highly precise delivered dose distributions. [227]

**Principles of Chemotherapy**

Early clinical trials evaluated the activity of single chemotherapeutic agents in children with recurrent or metastatic tumors. Few patients were cured with such an approach. The most active single agents that were identified in this manner were actinomycin-D, cyclophosphamide, vincristine, and doxorubicin. [228,229,230,231,232,233,234] and [235] Combinations of these agents led to improvements in response rates. [236,237] and [238] Wilburet al.[239] pioneered the administration of repetitive doses of vincristine, actinomycin-D (or dactinomycin), and cyclophosphamide (the VAC regimen) to children with advanced RMS. Of 24 children with inoperable localized tumors (16 patients) or metastatic deposits at diagnosis (8 patients), 16 (67%) were alive and well at a median follow-up approaching 2 years. [239] The benefit of systemic therapy on prolonging and improving overall survival was confirmed in numerous studies involving limited numbers of patients. [240,241,242,243,244] and [245]

Multicenter studies evaluating larger numbers of patients subsequently were initiated to ascertain better methods of treatment and to learn more about potential prognostic factors. In 1972, members of the Children's Cancer Study Group (CCSG) and the pediatric divisions of the Southwest Oncology Group and the Cancer and Acute Leukemia Group B banded together to form the IRS group. More than 3,500 patients with RMS and undifferentiated sarcoma have been treated on one of the four IRS investigations that have been completed since that time.[76,77] and [78,164] Accrual to IRS-V is expected to continue until 2002 or 2003. These studies have been instrumental in improving outcome, identifying important prognostic variables, and developing risk-based therapies for patients with RMS ( Fig. 32-8).

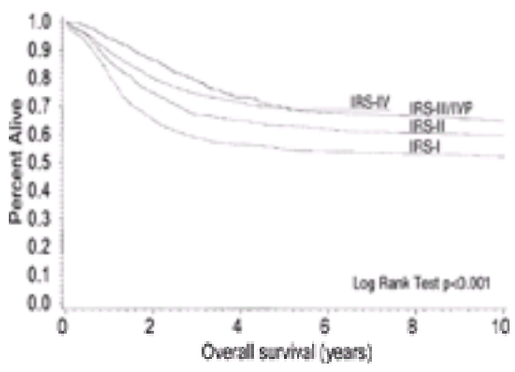


**FIGURE 32-8..** Survival of patients treated on Intergroup Rhabdomyosarcoma Studies (IRS)-I, -II, -III//IV-P (IV pilot), and -IV. Improvements were seen for each successive study from IRS-I through III/IV-P, but no significant improvement in outcome was seen with IRS-IV. (JR Anderson, *personal communication*, 2000.)

The goal of identifying additional active agents is to be able to incorporate as many active agents as possible, ideally without overlapping toxicities, into the frontline management of patients with newly diagnosed disease to avoid the development of multidrug resistance. [246,247] Cisplatin, etoposide, and dacarbazine have been shown to be active, singly and in various combinations, over the last two decades. [248,249,250,251,252,253] and [254]

Ifosfamide, alone and in combination with etoposide or doxorubicin, has been shown to be highly active in both newly diagnosed and recurrent RMS. [251,255,256] and [257] These observations formed the rationale for the basic experimental design of IRS-IV in which the efficacy of equitoxic doses [258] of cyclophosphamideversus ifosfamide (VAC) versus vincristine, actinomycin D, and ifosfamide (VAI) versus vincristine, ifosfamide, and etoposide (VIE) was evaluated.

One obvious problem with treating patients with recurrent tumors is that an agent's true activity may be underestimated grossly because such tumors are likely to have developed complex mechanisms of resistance to a broad spectrum of agents[259] and because such patients are less likely to tolerate treatment with sufficiently high doses of potentially active agents. In conjunction with a highly predictive murine xenograft model of RMS, [260,261] and [262] phase II window studies have been used to identify several agents with moderate to high activity, including melphalan, [263] methotrexate[264] and, most recently, topotecan,[265,266] which were “inactive” in previously treated patients.[252,267] Although a follow-up study by this same group failed to show a similarly improved response rate after dacarbazine and doxorubicin were administered to patients with newly diagnosed disease, [268] the concept of alternative induction therapy, consisting of the early introduction of pairs of active drugs, was

incorporated into the design of IRS-III for patients who had failed to achieve a complete response by week 20. The results of the three, two-drug, phase II window studies that were conducted in IRS-IV identified the combination of ifosfamide plus doxorubicin as a potentially significant drug pair for improving outcome in patients with metastatic RMS.[269,270]

More recently, the camptothecin analogs—topotecan and irinotecan—have been identified as particularly promising new agents. These compounds inhibit the DNA repair enzyme topoisomerase I and have both striking activity in the murine xenograft model[262] and encouraging responses in phase I and phase II window testing.[266,271,271a] Two major objectives of the IRS-V series of studies will be to evaluate the activity of irinotecan (given on a daily × 5 × 2 schedule) in patients with newly diagnosed metastatic RMS and to evaluate the efficacy of adding topotecan (on a daily × 5 schedule in combination with cyclophosphamide) to the standard three-drug VAC regimen for patients with intermediate-risk tumors.

Paclitaxel (Taxol) and its semisynthetic analog, docetaxel (Taxotere), are unique tubulin-binding compounds that produce cytotoxicity by blocking dividing cells at either the $G_2$ or the M phase of the cell cycle and have demonstrated significant antitumor activity against a variety of neoplasms.[272] Reports of antitumor responses in phase I studies in pediatric patients with recurrent solid tumors,[273,274] including those with RMS, have generated significant enthusiasm for additional clinical evaluation of these agents, used both singly and in combination with other drugs.[275,276] Both compounds are undergoing traditional phase II testing in treating RMS and other pediatric solid tumors, and phase II window testing also is planned. Finally, vinorelbine, a semisynthetic vinca alkaloid with unique pharmacologic principles, also was described recently as having promising activity in 17 patients with refractory and recurrent RMS or soft tissue sarcomas.[277]

**Combined-Modality Therapy**

The general principle of complete surgical removal of tumor, if feasible, should be emphasized. Patients whose tumors are removed at the outset continue to fare better than do those with gross residual sarcoma, especially with primary tumors of an extremity. Operative removal of residual tumor may be performed from weeks to months after completion of chemotherapy and RT and may help to eradicate resistant cells that otherwise may contribute to relapse.[278]

The timing of RT in relation to chemotherapy has been somewhat variable. RT was historically begun at the same time as chemotherapy for patients with group I or group II sarcomas but was delayed until week 6 for those with group III or group IV tumors to assess the response to chemotherapy, to exploit potentially the booster effect of certain drugs (e.g., actinomycin-D), and to minimize mucositis or other damage.[279,280] Currently, in the IRS-V series of studies, RT commences immediately for select high-risk patients with locally advanced cranial parameningeal tumors, at week 3 for appropriate patients on the low-risk study, at week 12 for patients on the intermediate-risk study, and at week 15 for patients on the high-risk study.

Because most relapses occur within 2 to 3 years of diagnosis, two to five chemotherapeutic agents historically have been administered for 12 to 24 months. The duration of therapy possibly may be shortened significantly without jeopardizing outcome as multi-agent regimens become increasingly risk-based and intensive. In IRS-IV, selected groups of patients with highly favorable prognosis (CG I and CG II patients with primary orbit or eyelid tumors and CG I patients with paratesticular tumors) received only 36 weeks of chemotherapy with just vincristine and actinomycin-D, and the duration of therapy for all other patients was reduced to approximately 1 year. Drugs, doses, and schedules vary among institutions. Select patients with extremely favorable prognoses (orbital and completely resected paratesticular tumors) generally can be treated safely without an alkylating agent (i.e., with just vincristine and actinomycin); other patients with “favorable” localized tumors (incompletely resected ERMS tumors arising in favorable locations and completely or gross totally resected ERMS tumors arising in unfavorable locations) generally will do well with the gold-standard three-drug regimen of VAC. Optimal therapy for patients with unresectable ERMS in unfavorable sites and for patients with ARMS and for those with metastatic tumors remains to be defined. Treatment guidelines from IRS-IV are summarized in Table 32-4. Treatment programs for RMS and undifferentiated sarcoma are still evolving, and the suggestions in Table 32-4 should not be considered definitive.

**TABLE 32-4. IRS-IV RECOMMENDATIONS FOR THERAPY FOR RHABDOMYOSARCOMA AND UNDIFFERENTIATED SARCOMA IN CHILDHOOD**

**Treatment Results**

Results of IRS-III are summarized in Table 32-5.[78] These results confirmed the major findings both of most other single and limited institution studies and of the earlier IRS series concerning the relation between outcome and the extent of initial surgical resection and site of the primary tumor.[76,77,241,242,245,281,282 and 283] Even after the difference in the distribution of patients by CG is taken into account, the overall outcome for patients treated in IRS-III was significantly better than that in IRS-II: 5-year progression-free survival (PFS), 65% ± 2% versus 55% ± 2% ($p$ <.001; Fig. 32-9). Most of the improvement in outcome was explained by the significantly improved results among selected groups of higher-risk patients, specifically those with CG III special pelvic tumors (5-year PFS, 74% ± 4% vs. 58% ± 5% in IRS-II; $p$ = .01); unfavorable histology CG I and CG II (71% ± 6% vs. 59% ± 5% in IRS-II; $p$ = .002); and CG III tumors excluding special pelvic, orbit, and selected head sites (61% ± 3% vs. 52% ± 3% in IRS-II; $p$ = .01; Fig. 32-10A). As compared with earlier series, outcome was improved for patients with localized tumors at virtually all sites, but no improvement in outcome was seen among patients with metastatic tumors. Outcome was best among patients with primary tumors of the orbit or nonbladder, nonprostate genitourinary tract; was intermediate among patients with tumors arising in other head and neck sites and in the bladder or prostate; and was worst among patients with extremity, cranial parameningeal, and other (trunk, pelvis-perineum, retroperitoneal, and paravertebral) sites (Fig. 32-10B). With the development of more effective doxorubicin-based systemic therapy and the use of local irradiation in all patients, alveolar histology was found to be associated no longer with adverse prognostic significance.

**TABLE 32-5. SUMMARY OF RESULTS FROM THE THIRD INTERGROUP RHABDOMYOSARCOMA STUDY (IRS-III) AND COMPARISONS, WITH IRS-II**

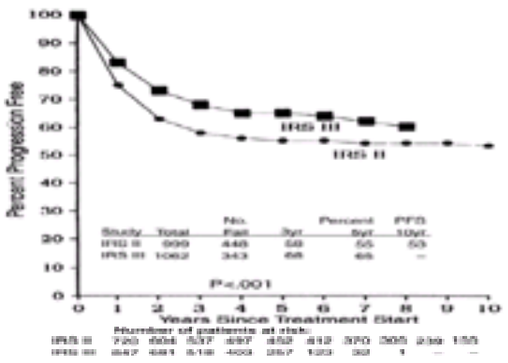


**FIGURE 32-9.** Progression-free survival (PFS) for all patients treated on Intergroup Rhabdomyosarcoma Studies (IRS)-II and -III. Even after adjustment for differences in clinical group distributions (resulting from changes in the primary surgical approach to certain tumors), the overall outcome of therapy in IRS-III was significantly better than in IRS-II (65 ± 2% vs. 55 ± 2%, *p* <.001). (From Crist W, Gehan EA, Ragab AH, et al. The Third Intergroup Rhabdomyosarcoma Study. J Clin Oncol 1995;13:610, with permission.)

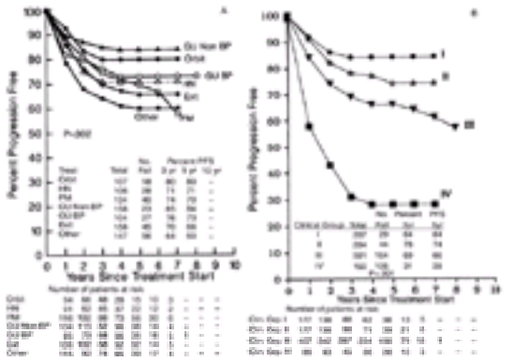

**FIGURE 32-10.** Progression-free survival (PFS) on Intergroup Rhabdomyosarcoma Study (IRS)-III by extent of initial surgical resection (clinical group) and site of primary tumor. **A:** Outcome according to site of primary tumor for patients with localized tumors (clinical groups I–III). Patients with localized tumors originating in nonbladder, nonprostate genitourinary sites (GU Non BP) or the orbit had the most favorable prognosis. In contrast to the results in IRS-II, patients with bladder or prostate tumors (GU BP) had outcomes that were comparable to those of patients with tumors in head and neck sites (HN). Patients with tumors arising in an extremity (Ext) or parameningeal (PM) or other site (e.g., retroperitoneum, trunk, pelvis, paraspinal region) fared least well. **B:** Outcome according to clinical group. Patients with completely (clinical group I) and gross totally (clinical group II) resected tumors did significantly better than did those with more advanced disease (clinical group III). Patients with metastatic disease (clinical group IV) fared significantly worse than all other patients. (From Crist W, Gehan EA, Ragab AH, et al. The Third Intergroup Rhabdomyosarcoma Study. J Clin Oncol 1995;13:610, with permission.)

One major question that was left unanswered by IRS-III and was not addressed in IRS-IV is the roles, if any, that cisplatin (with or without etoposide) and doxorubicin have in the frontline management of selected patients with RMS. As compared with the standard VAC regimen, the inclusion of cisplatin and etoposide did not appear to improve the complete response rate or PFS; however, differences in outcome among patients receiving doxorubicin were seen in two distinct groups of patients (Table 32-5). The inclusion of doxorubicin (with cisplatin) for patients with unfavorable histology CG I and CG II was associated with a significant improvement in outcome as compared with the results seen in IRS-II. It may ultimately be impossible to distinguish how much of this improvement was caused by the use of more effective systemic chemotherapy and how much by improved local control resulting from the uniform use of local irradiation.

The second group in whom treatment with doxorubicin was found to be associated unexpectedly with improved outcome consisted of patients with favorable histology CG II tumors, excluding orbit, head, and paratesticular sites. These patients were assigned randomly to receive regimen 33 (vincristine, actinomycin-D, and doxorubicin), and their 5-year PFS and overall survival rates were 77% ± 6% and 89% ± 5%, respectively. The IRS committee ultimately dismissed these findings after determining the absence of a significant difference between the results of regimen 33 and the combined results of the two vincristine–actinomycin-D regimens, regimen 32 in IRS-III and regimen 23 in IRS-II (5-year PFS and overall survival rates of 63% ± 8% and 73% ± 8%, respectively; *p*= not significant). However, the fact remains that a significant difference was seen when the findings of regimen 33 were compared only with the results among those patients who received regimen 32 and were treated concurrently (5-year PFS and overall survival rates of 56% ± 10% and 54% ± 13%; *p*= .08 and .03, respectively). The design of IRS-IV, in which doxorubicin is used only in one of three drug-pair phase II windows, did not include a follow-up evaluation of the role of doxorubicin in improving outcome for patients with limited-stage tumors, who appeared to benefit from the inclusion of doxorubicin in IRS-III.

Preliminary results of IRS-IV have begun to emerge in the last year and, with few exceptions, little to no improvement in outcome has been seen for most patients as compared to the results of IRS-III (Fig. 32-8). The major therapeutic objectives of IRS-IV were to compare randomly the efficacy of three regimens using three drugs—VAC, VAI, and VIE—and two schedules of RT (conventional and hyperfractionated). Three-year FFS figures for patients with low- or intermediate-risk RMS were 88% and 76%, respectively, not significantly improved from the results for comparable patients treated in IRS-III. [164] Outcome for patients with ARMS was unchanged as compared to that in IRS-III.[284] Subset analysis did, however, reveal two groups of patients with ERMS, representing approximately 25% of all patients with nonmetastatic tumors, for whom outcome in IRS-IV was improved.[164] Stage 1 patients with node-positive resectable or unresectable (CG III) tumors arising at certain favorable sites (head and neck but not orbit or eyelid and not cranial parameningeal) experienced an improvement in 3-year FFS from 72% in IRS-III to 92%. Three-year FFS was improved also for stage 2 and stage 3 patients (tumors arising in unfavorable sites) with completely or gross totally resected (CG I and CG II) tumors, increasing from 71% in IRS-III to 86%. The addition of an alkylator (cyclophosphamide or ifosfamide) to patients with group III orbital tumors produced a 3-year FFS of 100%.[285] Thus, although select groups of patients appear to have benefited from the increased alkylator intensity of IRS-IV, this strategy did not improve outcome for the majority of patients who present with either unresectable (group III) ERMS tumors arising in unfavorable locations (stages 2 and 3) or those with ARMS. For example, 3-year FFS among 139 patients with extremity RMS and treated in IRS-IV, 71% of whom had ARMS and 23% of whom had stage 4 disease, was 55%.[84] Again reflecting the clustering of clinical variables, the probability of treatment failure was greater than 50% in patients with large (5-cm), invasive (T2), node-positive (N1) unresectable (group III) tumors. None of the three-drug regimens was clearly superior to the others. Three-year FFS similarly was unchanged among patients with metastatic disease at diagnosis, for whom one of three phase II windows was employed: vincristine plus melphalan, ifosfamide plus etoposide, or ifosfamide plus doxorubicin. However, treatment on the vincristine plus melphalan arm appeared to be associated with a somewhat worse outcome than was treatment on either of the other two arms, perhaps owing to cumulative hematologic toxicity after early treatment with melphalan. [269,270]

The results of other international collaborative studies also have been published in the last several years. The International Society of Pediatric Oncology treated 186 patients with newly diagnosed nonmetastatic RMS in its MMT-84 study.[286] Treatment consisted of three courses of IVA for patients with completely resected tumors and six to ten courses of IVA for those with incompletely resected tumors. The major difference in therapeutic approach between the MMT-84 study and the IRSG studies was the omission of RT (or second-look surgery) for patients achieving a complete remission with chemotherapy alone; radiation was, however, given to patients younger than 5 years having parameningeal tumors, reflecting the recommendations of an international workshop on the management of such tumors, [215] and to patients older than 12 years having tumors in any site. With a median follow-up of 8 years, the 5-year event-free survival (EFS) was 53%, and overall survival was 68%. With the exception of the excellent outcome (5-year EFS of 85% ± 12%) among the small number of patients (n = 27) having completely resected tumors and receiving three cycles of VAI over 2 months, EFS in all other groups was inferior to that of similar-risk patients treated in IRS-III and IRS-IV, largely owing to a significantly greater risk of local relapse. Nearly one-third of patients (54 with local tumors only, 7 with local plus distant tumors) experienced an isolated local relapse, and most subsequently did not achieve long-term disease-free status even after the application of aggressive local RT or surgery (or both). As in the IRSG studies, factors associated with improved prognosis included embryonal histology (56% 5-year EFS vs. 33% for patients with ARMS; *p* = .001) and favorable site (orbit and genitourinary/nonbladder-nonprostate sites). Although the intent of avoiding the late effects of potentially “unnecessary” RT was laudable, the inability to salvage the majority of the large number of patients with disease that relapses with this approach suggests that it represents a suboptimal strategy for the vast majority of patients

with newly diagnosed RMS.

The German Soft Tissue Sarcoma Study (CWS-86) treated 310 eligible patients, including 286 with RMS (251 with localized disease), with four-drug chemotherapy (vincristine, doxorubicin, ifosfamide, and actinomycin-D) for varying periods (ranging from 16 to 40 weeks) based on the initial extent of disease. [287] The use of local RT was stratified by stage of disease, primary tumor site, response to neoadjuvant chemotherapy, and the results of second-look surgery: "Good responders" received 32.0 Gy, whereas "poor responders" received 54.5 Gy in split courses of accelerated hyperfractionation. Children younger than 2 years did not receive RT, and those between the ages of 2 and 3 did so on a case-by-case basis. More than one-half of all patients had tumors that were unresectable or metastatic (or both) at diagnosis. Five-year EFS for all RMS patients was 60% ± 3% and, for those without metastases, it was 68%. As in the IRSG and the International Society of Pediatric Oncology studies, outcome was related closely to stage of disease at diagnosis, ranging from 83% in patients with stage I disease (n = 46) to 67% in patients with stage II disease (n = 56) to 63% in patients with stage III disease (n = 149). The risk of local relapse clearly was related to failure to administer consolidative local RT in patients with either positive margins or gross residual disease. Better outcome was seen among the patients with ERMS than among those with ARMS (67% ± 4% vs. 41% ± 6%; *p*= .01) and among patients with genitourinary (nonbladder and nonprostate) tumors. Other factors that were found to correlate with improved outcome differed according to stage at diagnosis: Among patients with stage I disease, only tumor size of at least 5 cm was associated with an adverse outcome, whereas for patients with stage III tumors, female gender, tumor size of at least 5 cm, alveolar histology, and all sites other than genitourinary (nonbladder and nonprostate) were associated with poorer outcome.

Smaller, limited, and single-institution pilot studies, primarily of patients with intermediate-risk RMS, have achieved encouraging results using five-drug chemotherapy consisting of alternating cycles of vincristine, doxorubicin, and cyclophosphamide and ifosfamide and etoposide. Arndt et al. [288] treated 30 patients with intermediate-risk tumors (14 with parameningeal primary tumors, 6 with extremity primary tumors, 5 with bladder or prostate tumors, 12 with ARMS, and 2 with undifferentiated sarcoma) with 14 cycles vincristine, doxorubicin, and cyclophosphamide alternating with ifosfamide and etoposide; most patients received 5,040 cGy for local control beginning immediately after week 12 and cycle 5 of chemotherapy. [288] Three-year EFS in this group of high-risk patients with nonmetastatic tumors was 85% (95% confidence interval, 72% to 99%). Similar encouraging results were seen in 15 patients with intermediate-risk RMS treated on granulocyte colony-stimulating factor–supported "interval compression" variation of the same basic five-drug regimen. [289] Although the results of these nonrandomized pilot studies cannot be compared directly to those seen in IRS-IV and other international cooperative group studies, they nonetheless lend support to the observation made in IRS-III that the use of doxorubicin may improve outcome for select groups of intermediate-risk patients (unfavorable site or histology, or both, and unresectable disease).

For patients at high risk of treatment failure, primarily those with metastatic disease at diagnosis, consolidation with high-dose chemotherapy and autologous bone marrow or peripheral blood stem-cell rescue (so-called bone marrow or stem-cell transplantation) has been studied as a way of improving outcome by overcoming intrinsic or acquired drug resistance through delivery of what otherwise would be "lethal" doses of chemotherapy (generally including one or more alkylating agents)—with or without total-body irradiation—and then "rescue" of the patient by infusing previously cryopreserved hematopoietic progenitor cells. A series of small studies, with differing eligibility criteria, different myeloablative regimens, and different subsets of patients with metastatic disease have failed to demonstrate any significant benefit of such transplantation in this setting ( Table 32-6).[282,290,291,292,293 and 294] Thus, this strategy, although still being pursued, must be considered of no proven benefit and highly experimental.

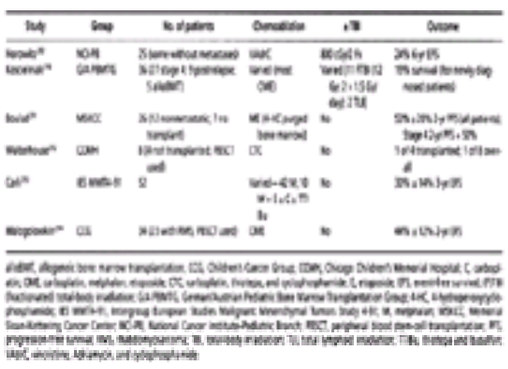

**TABLE 32-6. SUMMARY OF RESULTS OF MEGATHERAPY CONSOLIDATION WITH AUTOLOGOUS HEMATOPOIETIC BONE MARROW AND STEM-CELL SUPPORT FOR PATIENTS WITH RHABDOMYOSARCOMA**

## Complications of Therapy

Complications of therapy include residual effects of operative management, RT, and chemotherapy (see also Chapter 10, Chapter 12, Chapter 13, and Chapter 49). Clearly, the extent of surgical treatment has a bearing on the functional outcome. Extremity amputations and extensive formal lymph node dissections are performed rarely for that reason. Infection, anemia, and bleeding can result from intensive drug combinations. Infants may develop more toxicity than do older children. [295] Leukoencephalopathy, which is very uncommon, can be seen in patients with cranial parameningeal sarcoma given intensive chemotherapy and RT, including repeated doses of intrathecal methotrexate, hydrocortisone, and cytosine arabinoside. [296] Ascending myelitis of the cervicothoracic region has been observed in 5 of 149 children treated between 1972 and 1984 with intensive chemotherapy and irradiation for high-risk cranial parameningeal sarcoma. [297] Fibrosis, diminished growth of underlying or surrounding structures, cataracts, disturbed dentition, and growth hormone deficiency have been reported in children receiving multimodality therapy for tumors arising in the head and neck.[298,299] Combined therapy with drugs and irradiation can produce severe fibrosis and limitation of function in irradiated sites. For orbital tumors, the severity of radiation-related complications may be reduced by the avoidance of simultaneous administration of irradiation and sensitizing chemotherapy (e.g., actinomycin-D or doxorubicin).[209]

With modern therapy, a minority of patients with tumors of the bladder or prostate require total or partial cystectomy. [78,300] Nonetheless, approximately 25% of those who do retain their bladders suffer from significant bladder dysfunction (incontinence, frequency, nocturia), and all patients with bladder or prostate tumors are at risk for hematuria, structural renal abnormalities, and delayed growth and pubertal development requiring sex hormone replacement therapy. [301,302] The incidence of hematuria after cyclophosphamide therapy can increase as much as fourfold if pelvic irradiation is used, but the use of the bladder protectant mesna has all but eliminated this potentially life-threatening complication. [303,304] Infertility can result from gonadal effects of cyclophosphamide or RT or from surgical interruption of nerves conveying impulses for antegrade ejaculation, as in boys with pelvic or paratesticular sarcoma. [305]

Several reports have described a broad spectrum of late complications, including small-bowel obstruction, esophageal and common bile duct strictures, inguinal nerve entrapment syndrome, and lymphedema of the leg in patients treated with multimodality therapy for paratesticular RMS. [306,307] Among the newer active agents, ifosfamide can cause renal tubular dysfunction, which may be severe, with aminoaciduria, glycosuria, cation leakage, increased serum creatinine, and acidosis with growth failure.[308] Renal toxicity data recently were reported for 194 previously untreated patients enrolled in one of two ifosfamide-containing pilot regimens in IRS-IV.[309] The overall incidenceof any nephrotoxicity was 14% (28 patients), with the most frequent toxicities being renal tubular dysfunction and decreased glomerular function. Preexisting renal abnormalities (primarily hydronephrosis) and age younger than 3 years (especially for those who received a cumulative ifosfamide dose of more than 72 g per $m^2$) greatly increased the risk of renal tubular dysfunction.

The most devastating late complication of therapy is the development of a second malignant neoplasm. Twenty-two of these neoplasms developed among 1,770 patients entered into IRS-I and IRS-II, including 11 radiation-related bone sarcomas and 5 cases of acute nonlymphoblastic leukemia, at a median of 7 years after therapy.[310] The observation that three of the affected patients had neurofibromatosis and that the families of seven of the affected patients had histories compatible with LFS raises the possibility that genetic susceptibility plays a significant role in the development of a second malignant neoplasm after treatment for RMS. Early results from IRS-III described the early occurrence of five cases of acute myeloid leukemia (AML) in children and one case of osteosarcoma and one case of myelodysplastic syndrome (MDS).[311] A preliminary report of second malignant neoplasm in IRS-IV found 14 cases in 13 patients at a median of 3.2 years from diagnosis.[312] The 7.2% riskof secondary leukemia (MDS/AML and acute lymphatic leukemia) in patients treated with melphalan (as part of the phase II window strategy for patients with metastatic disease) was significantly greater than that for all other treatment arms. Early concerns about an increased risk of AML/MDS in patients receiving etoposide did not appear to be substantiated; however, prospective monitoring of the contribution of a strong family history of cancer to the risk of

developing a treatment-related second malignant neoplasm is prudent. [313]

The development of risk-based therapies clearly is the most important strategy to minimize the risk of late treatment-related complications. As was demonstrated in IRS-III and IRS-IV and is being evaluated further in IRS-V and in studies in several European countries, selected groups of patients with favorable prognosis can be identified; for them, the elimination of one or more chemotherapeutic agents does not appear to affect ultimate outcome adversely. Although this approach does not appear to have been successful for special pelvic tumors, possibly individual components of the multimodality treatment regimens can be withheld from the frontline management of patients without compromising ultimate outcome. Such an approach has been tried for patients with orbital RMS and, despite a local recurrence rate of 45% in patients not receiving initial RT, long-term survival does not appear to have been compromised. [208]

Patients with adverse prognostic features are being treated increasingly with more intensive and complex therapies. As therapy for even high-risk patients becomes more successful, it is increasingly important to monitor these patients for the development of other, potentially life-threatening, late treatment-related adverse events and to understand better the relation between genetic susceptibility and the development of second malignant neoplasms. Identifying people for whom the selective elimination of one category of antineoplastic agents (e.g., epipodophyllotoxins, alkylating agents) is appropriate may be possible because of the presence of high-risk genetic markers. Improvements in evaluation of the presence or absence of microscopic (residual) disease (e.g., with the use of molecular genetic markers unique to the tumor cell) may permit individualization of the duration of therapy. The goal of all these therapeutic maneuvers is the identification of better treatments (i.e., treatments that achieve maximum long-term survival with minimum short- and long-term morbidity).

## Treatment of Recurrent Disease

The management of patients with recurrent or unresponsive sarcoma is problematic. Although rare after 3 or 4 years from diagnosis, recurrence can take place many years after apparently successful treatment.[314,315] and [316] Recurrence connotes relative or absolute resistance to the chemotherapy or irradiation used in initial therapy. Among the various molecular mechanisms that may play a role in the development of drug resistance are overexpression of P-glycoprotein (the mediator of the multidrug resistance phenotype), hypermethylation of the tumor cells' DNA, elevated levels of DNA polymerase-a and -b and topoisomerase II, overexpression of O6-methylguanine-DNA methyltransferase, and constitutive expression of the c-H-*ras* oncogene.[317,318,319,320,321,322,323] and [324]

The optimal treatment of patients with recurrent tumor is, at best, imprecisely defined. Patients with suspected recurrent disease must be evaluated fully and staged before the formulation of a treatment plan. Recurrence always should be documented by biopsy or fine-needle aspiration. At a minimum, imaging studies (plain films, computed tomography, or magnetic resonance imaging) should be performed to evaluate the lungs, site of primary tumor, and any sites suggested by the history and physical examination. Hematologic abnormalities should be followed up with a bone marrow biopsy. After pathologic verification of recurrent disease, factors that should be considered in the formulation of a treatment plan include the timing of the recurrence relative to the completion of therapy (i.e., progression or recurrence while on therapy, within the first 6 to 12 months off therapy, or after more than 12 months off therapy), the extent of disease at recurrence (localized vs. disseminated), the extent of disease at diagnosis, and the nature of prior therapy (extent and intensity of chemotherapy, sites and doses of previous irradiation). Achieving long-term survival is difficult, particularly in patients whose disease progresses on therapy or shortly after treatment completion and in patients who initially had unresectable or metastatic sarcoma.[279] The 3-year post-relapse survival rates for patients treated in IRS-III were 48% ± 12%, 12% ± 9%, 11% ± 5%, and 8% ± 4%, for patients with CG I, II, III, and IV disease, respectively ($p$ <.001 in favor of CG I).[78]

Several recent reports have better defined the risk of death from disease after relapse. Post-relapse survival was analyzed in 605 children who previously had been treated in IRS-III, IRS-IV pilot, and IRS-IV (1984 to 1997). [325] Ninety-five percent of all relapses occurred within 3 years of diagnosis; the latest reported recurrence was at 9 years. The median survival time from first recurrence was 0.8 years; fewer than 20% of patients were projected to be alive 5 years after relapse. Post-relapse treatment was nonuniform. Subset analyses were able to identify groups with different prognoses. Approximately one patient in five, primarily those with ERMS who initially presented with stage 1 or group I disease, had a relatively “favorable” 5-year survival rate of approximately 50%, and the likelihood of subsequent survival appeared to correlate with whether the relapse was local (72%), regional (50%), or distant (30%). The estimated 5-year post-relapse survival for patients with stage 2/3 or group II/III tumors was 20%, and for those with group IV disease, it was 12%. For this latter group of patients, the type of relapse (local vs. regional vs. distant) was not prognostically relevant. Patients with recurrent ARMS had a worse prognosis, with only those who initially had group I disease enjoying a relatively favorable prognosis (40% 5-year survival vs. 3% for groups II to IV). Although potentially important treatment variables existed, similar findings were reported by the CWS group.[326] Forty-four patients (17 with ERMS, 13 with ARMS) with relapsed soft tissue sarcomas were treated with multi-agent chemotherapy; 5-year EFS appeared to be better in patients with ERMS (41% ± 12%) than in those with ARMS (23% ± 12%). The administration of post-relapse irradiation (presumably given to those who previously had not been irradiated and, therefore, were at greater risk of locoregional relapse after presenting with limited-stage disease) was associated with improved outcome.

### *Localized Recurrence*

An aggressive multimodality approach should be considered for patients with an isolated site of recurrent disease. The best chance for long-term survival is a situation in which a complete surgical resection can be accomplished, potentially followed by adjuvant postoperative irradiation and chemotherapy, in patients with an initial stage 1 or group I ERMS tumor. For example, for patients with vaginal and paratesticular localized recurrences, long-term survival still can be achieved with this aggressive approach.[327] The choice of a salvage chemotherapeutic regimen depends on the previous chemotherapy and the timing of the relapse. There are well-described examples of durable responses achieved in patients with recurrence off therapy using the same drugs as those previously administered. [328] For patients who have not previously received known active agents, such as ifosfamide, doxorubicin, or etoposide, or such newer agents as topotecan or irinotecan, these agents should be considered strongly in choosing a regimen, although the tolerability and activity of these agents are likely to be reduced in heavily pretreated patients. [329] In patients who already have received all known active agents and who have recurrence during or shortly after completion of therapy, investigational phase I and phase II agents should be considered.

### *Disseminated Recurrence*

In contrast to localized recurrence, in which the chance of long-term survival is a small but real possibility, recurrence that develops with metastatic disease is essentially incurable. Surgical resection of metastatic lesions, even if complete, is unlikely to be of benefit with regard to curative potential, although low-morbidity procedures may be useful for palliative purposes.[189,330,331] and [332] Chemotherapy is the primary therapeutic modality, with palliative RT usually reserved to treat painful lesions or to prevent spinal cord compression. The choice of chemotherapeutic agents should be guided by the same principles listed previously.

Although high-dose chemotherapy with autologous bone marrow or peripheral blood progenitor cell support has not been demonstrated to be efficacious in the setting of patients with newly diagnosed metastatic disease, the efficacy of programs incorporating these strategies for children with recurrent soft tissue sarcoma is unclear, because few studies have been undertaken and, with rare exceptions, only short-term results are available. [333,334] and [335] Because soft tissue sarcomas usually are less sensitive to irradiation than are leukemia or neuroblastoma cells, total-body irradiation may be less effective in eradicating recurrent sarcoma. [282] Further investigation of consolidation with high-dose chemotherapy and bone marrow or peripheral blood stem-cell rescue for select patients with recurrent RMS is not unreasonable, given the current lack of good therapeutic options. One possible advantage of such an approach, particularly in heavily pretreated patients who are relatively intolerant of additional myelosuppressive chemotherapy, is to use growth factor–supported autologous peripheral blood progenitor cell transfusions to permit repetitive monthly cycles of myeloablative chemotherapy.[336]

## Immunotherapy and Other Biologic Interventions

Recent advances in understanding the basic biology of RMS have led to the possibility of novel therapeutic interventions, including specific antitumor immune responses and interruption of autocrine growth factor loops. The recognition that intracellular proteins can be processed and presented as peptides on the cell surface by major histocompatibility complex class I molecules has suggested the possibility that tumor-specific mutant gene products may be targets for cytotoxic T cells.[337,338] For example, investigators have shown that a peptide derived from a mutant p53 protein is recognized specifically by cytotoxic T cells. [339,340] In a similar way, translocation-specific fusion proteins also could be targeted potentially by cytotoxic T cells. Specifically, the PAX-FKHR fusion protein generated by the t(2;13)(q35;q14) translocation in ARMS is a potential target for cytotoxic T cells therapeutic approaches. Pilot clinical studies using PAX-FKHR specific peptide, pulsed, dendritic cell vaccinations are ongoing (C. Mackall et al., *personal communication*, 2000). The success of this approach will depend on the ability of tumor cells to present a processed fusion peptide bound to major histocompatibility complex on the cell surface. If this can occur, multiple approaches could be taken to overcome potential deficits that allowed the tumor initially to escape cellular immunity. [341,342]

Because an IGF-2 autocrine pathway has been demonstrated to play a role in the growth of RMS, [30,31] disruption of this pathway offers another potential therapeutic target. Blockade of the IGF-1 receptor, a ligand-inducible tyrosine kinase receptor that mediates the IGF-2 mitogenic signal, has been shown to inhibit the growth of established human RMS tumors in nude mouse xenografts.[30] These experiments used mouse monoclonal antibodies, which generally have not proven to be of clinical utility. However, the rapid development of human antimouse antibodies allows for the generation of humanized monoclonal antibodies, which holds some promise for the clinical application of this approach, as has been demonstrated using several antibodies now in routine clinical practice (e.g., including Herceptin and Rituxan).[343,344] Of further interest, recent reports have suggested that antibodies directed against a growth factor receptor may act synergistically with standard cytotoxic chemotherapy.[345,346 and 347] Indeed, a variety of compounds designed to block specific growth factor pathways now are in preclinical or clinical development. [348] Compounds with tyrosine kinase or farnesyl transferase inhibitory activity currently are being developed in the hope that these compounds may be able to block specific growth factor or oncogene pathways, respectively. [349,350]

A relatively new approach to treatment of many solid tumors that has shown great promise in model systems is the use of angiogenic inhibitors. Compounds with inherent anti-angiogenic activity, including endostatin and angiostatin, [351] and agents inhibiting pro-angiogenic signaling, such as humanized vascular endothelial growth factor antibodies,[352] currently are in various stages of development. Although these agents have not been tested directly against RMS, other anti-angiogenic compounds have been reported previously to have activity against RMS xenografts. [353] Finally, camptothecin (irinotecan) and topotecan, agents currently in clinical trial in patients with RMS, have been demonstrated to have significant anti-angiogenic activity, raising the possibility of achieving an “accidental” inhibitory effect, particularly with protracted low-dose drug administration. [354,355]

## PERSPECTIVES

The published results of the IRS-IV study now are beginning to appear, and accrual of patients in the three IRS-V studies probably will continue for 2 more years. Great progress has been made in the treatment of RMS, and most patients with nonmetastatic tumors now are cured. These improvements have been based largely on a foundation of empiric observations achieved in the setting of well-designed clinical studies rather than basic insights resulting from epidemiologic and laboratory studies of the underlying biology of RMS. Although important clues now are being discovered correlating distinct molecular abnormalities with both pathogenesis and prognosis, a plateau appears to have been reached, particularly for the majority of patients who present with unresectable ERMS in unfavorable sites and for those with ARMS of any site. Improvements in both local and systemic disease control still are needed for these groups of patients. Local and locoregional recurrences continue to represent an unacceptably high proportion of all treatment failures. Although better ways of preserving the bladder and female genitourinary structures have become the norm, newer treatment modalities are needed to lessen the late adverse effects on structures in the head and neck.

As new agents with antisarcoma activity become available, possibly RT can be reduced still further (e.g., in patients with orbital lesions). Given the constraints of study design and the relatively small differences that may be expected for patients with localized tumors with favorable prognosis, future clinical studies probably will continue to focus on questions of therapeutic equivalence (i.e., treatments that maintain excellent outcome while reducing the short- and long-term complications of therapy). For patients with intermediate-risk tumors, including the majority of those with unresectable ERMS arising in unfavorable locations and patients with ARMS, the current IRSG strategy to improve outcome includes the evaluation of the efficacy of adding the two-drug combination of topotecan and cyclophosphamide to standard VAC chemotherapy and the role of the more aggressive use of delayed surgical resection in patients with initially unresected tumors.

Single investigational drugs or drug pairs are being assessed increasingly in the setting of a phase II window before introduction of standard therapy in patients with newly diagnosed metastases. This approach will continue in the high-risk arm of IRS-V where, currently, the activity of irinotecan is being evaluated. Although this strategy has been shown to be useful in identifying highly active compounds, it is doubtful whether the addition of conventionally dosed supplemental agents with similar mechanisms of action will improve the outlook significantly for patients with metastases at diagnosis. Significant improvements in outcome probably will not be achieved in such patients until the current generation of etiologic and epidemiologic studies, as well as tumor biologic studies exploring basic molecular mechanisms of tumorigenesis and metastatic spread, begin to yield their fruit.

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33

# EWING'S SARCOMA FAMILY OF TUMORS: EWING'S SARCOMA OF BONE AND SOFT TISSUE AND THE PERIPHERAL PRIMITIVE NEUROECTODERMAL TUMORS

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## INTRODUCTION

Ewing's sarcoma of bone originally was described in 1921 by James Ewing,[1] who characterized it as a tumor of the shaft of long bones that, in contrast to osteosarcoma, was sensitive to radiation. Ewing speculated that this tumor was of endothelial origin, an idea that prevailed until the mid-1980s. Recent evidence reviewed in this chapter confirms a neural origin for Ewing's sarcoma. Although Ewing's sarcoma most commonly is an undifferentiated tumor of bone, it may arise also from soft tissues [extraosseous Ewing's sarcoma (EES)]. A more differentiated form of this entity, known as *peripheral primitive neuroectodermal tumor* (PPNET), or *neuroepithelioma*, occurs as a primary tumor of bone or soft tissues. Both Ewing's sarcoma and PPNET share the same histochemical staining profile and a unique nonrandom translocation. This chapter provides an integrated discussion of the Ewing's sarcoma family of tumors (ESFT), which is now accepted to be a spectrum of a single neoplastic entity.[2]

The discussion in this chapter uses specific terminology. *Ewing's sarcoma* is a tumor of that histology of bone or soft tissue origin. Ewing's sarcoma of bone is a primary bony tumor, and extraosseous Ewing's sarcoma is a soft tissue tumor. PPNET is synonymous with peripheral neuroepithelioma and may be of bone or soft tissue origin. A general term is needed to refer to any of these tumors arising in any site, because most of the literature does not make these relatively recent histologic distinctions. Also, the clinical features of the diseases probably relate more to their primary sites and extent than to their histologic differences. The general term we have chosen is *Ewing's sarcoma family of tumors*.

## EPIDEMIOLOGY

ESFT (Ewing's sarcoma and PPNET) are the second most common primary osseous malignancy in childhood and adolescence. Classically, they are believed to originate in bone, although these tumors can occur also in soft tissue. The annual incidence in the United States is 2.1 cases per million children.

ESFT occur most commonly in the second decade of life. Nearly one-half of all patients with ESFT are between 10 and 20 years of age, and 70% are younger than 20. ESFT rarely develop in adults older than 30 years or in very young children.[3,4 and 5] As with many pediatric tumors, ESFT show a slight male predominance.

Racial and ethnic factors are important in Ewing's sarcoma. Particularly, the ESFT affect whites and are extremely rare among blacks in the United States and in Africa.[6,7 and 8] These tumors have been reported in India and Japan but are distinctly uncommon in China.[9]

The ESFT have not been associated with a familial cancer syndrome.[10] The risk of malignancy in mothers of children with Ewing's sarcoma is not in excess of that expected.[10,11] The ESFT have been reported in siblings, although the incidence is very low.[12,13] One study did show a significant increase in neuroectodermal tumors and stomach cancers in families of patients with Ewing's sarcoma.[14] The ESFT are not commonly associated with other congenital diseases of childhood. Reports of association with skeletal abnormalities (e.g., endochondroma, aneurysmal bone cyst), genitourinary abnormalities (e.g., hypospadias, reduplication of the renal collecting system), Down syndrome, and hereditary retinoblastoma exist but are uncommon, suggesting chance occurrences rather than a biologic basis.[15,16] Other than a single report of trisomy 21, other constitutional chromosomal abnormalities have not been reported in patients with the ESFT.

In terms of environmental factors in the etiology of Ewing's sarcoma, a recent case-control study of 153 children with Ewing's sarcoma did not identify any important environmental or familial risk factors for the development of this neoplasm.[17] Moreover, radiation exposure does not appear to be a common cause of Ewing's sarcoma. For example, no increase in the incidence of Ewing's sarcoma was reported after exposure to nuclear fallout in Japan.[18] In a large study of secondary bone tumors after radiotherapy, 3% were identified as Ewing's sarcoma, whereas 69% of the tumors were diagnosed as osteosarcoma.[19]

The tumor may occur as a second malignant neoplasm; however, the incidence is fairly low. Ewing's sarcoma has presented as a second malignant tumor in previously irradiated areas and in nonirradiated areas.[20,21]

## BIOLOGY

## Histogenesis

Since the initial description of this unique bone and soft tissue neoplasm as a diffuse endothelioma, [1] considerable effort has been expended in an attempt to define the cell of origin. Although the histogenetic origin of Ewing's sarcoma has been ascribed over the decades to endothelial, mesenchymal, and hematopoietic stem cells,[22,23,24,25,26 and 27] recent evidence from immunocytochemical, cytogenetic, and molecular genetic investigations indicates a neural crest origin for the ESFT.[28,29,30,31,32,33,34,35,36 and 37]

Derivation from a primitive, pluripotent, neural crest cell line is supported by the fact that these tumors synthesize acetylcholine transferase, which is essential to acetylcholine synthesis.[29,30,36] Because of the ability to synthesize acetylcholine transferase and the lack of appreciable synthesis of adrenergic pathway precursors, the ESFT are believed to be derived from postganglionic parasympathetic primordial cells located throughout the parasympathetic autonomic nervous system. The variety of soft tissue and bony locations may be explained in part by the wide distribution of these pluripotent stem cells throughout the parasympathetic autonomic nervous system. In contradistinction, neuroblastomas synthesize neurotransmitters associated with the sympathetic autonomic nervous system. [29,30] The origin of neuroblastoma is believed to be from adrenergic or mixed cholinergic-adrenergic primordial cells composing the sympathetic nervous system, including the adrenal medulla and paraspinal ganglia. In addition, Ewing's sarcoma and PPNET produce a different insulin-like growth factor-1 (IGF-1) than that synthesized by neuroblastoma (IGF-2).[38,39 and 40] IGF receptor is expressed in Ewing's sarcoma and allows for a self-stimulatory (autocrine) growth loop, with the IGF-1 produced by the neoplastic cells binding to their own cell-surface receptors. Similarly, gastrin-releasing peptide and its receptor are found with Ewing's sarcoma but not with other small round-cell tumors of childhood, including neuroblastoma. [41] This provides another autocrine growth mechanism, ensuring continuous growth in Ewing's sarcoma. Gastrin-releasing peptide is further proof of a neuroectodermal origin, because this peptide normally is expressed in brain and neuroendocrine cells in the lungs, pancreas, and gastrointestinal system. It is an autocrine growth factor in small cell lung carcinoma and other neuroendocrine malignant tumors. Unlike neuroblastomas, which may show amplification of N-*myc*, the ESFT do not amplify N-*myc*; rather these tumors possess high levels of c-*myc* RNA with or without amplification.[42,43 and 44] These differences in histogenesis between the ESFT and neuroblastoma are supported by cytogenetic, proto-oncogene, histopathologic, ultrastructural, and immunophenotyping data.

Although atypical Ewing's sarcoma and PPNETs (peripheral neuroepithelioma, peripheral neuroectodermal tumors, and Askin tumor) possess certain neural features, as demonstrated by the presence of Homer Wright rosettes, neural processes, neurosecretory granules, and neural immunocytochemical markers, “classic” Ewing's sarcoma has been defined as a tumor composed of primitive cells purportedly lacking neural differentiation. [45] However, the parasympathetic nervous system origin is supported by the ability of cell culture lines derived from undifferentiated Ewing's sarcoma tumors to undergo neural differentiation. [30,33,34,35,36 and 37] Undifferentiated Ewing's sarcoma cell lines develop neural processes, neurosecretory granules, and neurofilament triple proteins and secrete neurosecretory granules when induced by various agents (serum-depleted medium, tissue plasminogen activator, retinoic acid, nerve growth factor, and dibutyryl cyclic adenosine monophosphate). After exposure to these neural inducing agents, undifferentiated Ewing's sarcoma cell lines have been shown by phase-contrast microscopy and both transmission and scanning electron microscopy to possess branching neurite-like processes with varicosities, neurofilaments, and electron-dense neurosecretory granules. [33,34,35,36 and 37] With transfer into a typical serum-based growth media without inducing agents, the “differentiated” cell lines revert to their more primitive forms. Neural differentiation is inhibited by phorbol myristate acetate, a putative tumor promoter.[33] In addition, Ewing's sarcoma cell lines possess receptors for neuropeptide Y, a parasympathetic regulatory polypeptide.[32] Furthermore, both b-adrenergic and dopamine D-1 receptors have been identified in cultured Ewing's sarcoma cell line. [46] Cholecystokinin expression also is present and suggests that these cells originate from a postganglionic parasympathetic progenitor. [29,30] The neural crest parasympathetic origin is supported further by the fact that Ewing's sarcoma cell lines rapidly incorporate choline into acetylcholine and phosphorylcholine. [36] Morphologically undifferentiated Ewing's sarcoma cell lines are capable of acetylcholine synthesis, express markers for synaptic vesicles, and possess proteins associated with calcium-dependent release; however, these cell lines lack an organized mechanism for acetylcholine release. [36] This information and cytogenetic, proto-oncogene, and certain immunocytochemical similarities provide evidence that the ESFT represent a continuum from typical undifferentiated (classic) Ewing's sarcoma to atypical poorly differentiated Ewing's sarcoma to differentiated PPNET (peripheral neuroepithelioma, peripheral neuroectodermal tumor).

## Cytogenetics and Molecular Genetics

Incredible advances have been made in defining soft tissue tumors on the basis of chromosomal translocations, employing both cytogenetic and molecular genetic techniques. Recurring, nonrandom chromosomal translocations and fusion genes have been identified for alveolar rhabdomyosarcoma [t(2;13)(q35;q14), PAX3-FKHR; t(1;13)(p36;q14), PAX7-FKHR]; neuroblastoma [t(1p36;17q)]; malignant melanoma of soft parts [t(12;22)(q13;q12), EWS-ATF1]; desmoplastic small round-cell tumor [t(11;22)(p13;q11-12), EWS-WT1]; myxoid liposarcoma [t(12;16)(q13;p11), TLS-CHOP; t(12;22)(q13;q12) EWS-CHOP]; synovial sarcoma [t(X;18)(p11.2;q11.2), SYT-SSX-1, and SYT-SSX2, t(5;18)(q11;q11), SYT-Unknown)]; extraskeletal myxoid chondrosarcoma [t(9;22)(q22;q12), EWS-CHN]; congenital fibrosarcoma and mesoblastic nephroma [t(12;15)(p13;q25), ETV6-NTRK3]; dermatofibrosarcoma protuberans [t(17;22)(q22;q13), COL1A1-PDGFB]; hemangiopericytoma [t(12;19)(q13;q22)]; and others.[47,48,49,50,51,52,53,54,55,56,57,58,59,60 and 61] Likewise, the ESFT that include Ewing's sarcoma, PPNET, and Askin tumor express five reciprocal translocations (Table 33-1), with EWS-FLI1 [t(11;22)(q24;q12)] being most frequent. This chromosomal translocation (EWS-FLI1) is present in 90% to 95% of tumors within the Ewing's sarcoma family. The second most common translocation is EWS-ERG [t(21;22)(q22;q12)], which occurs in 5% to 10% of tumors. Rarely, Ewing's sarcoma will express three other translocations, including EWS-ETV1 [t(7:22)(p22;q12)], EWS-EIAF [t(17;22)(q12;q12)], and EWS-FEV [t(2;22)(q33;q12)]. In addition to these tumor-defining chromosomal translocations, other complex nonrandom translocations are associated with this family of tumors[60,61,62,63 and 64] and include [t(11;14;22)(q24;q11;q12)], [t(10;11;22)(p11.2;q24;q12)], [t(11;17;22)(q24;q22;q12)], [t(11;18;22)(q24;q22;q12)], and [t(4;11;22;)(q21;q24;q12)]. As noted, the majority of variant or complex translocations in the ESFT includes EWS-FLI1 [t(11;22) (q21;q12)]; however certain tumors have been shown to have the chromosomal translocations EWS-ERG, EWS-ETV1, EWS-EIAF, and EWS-FEV, which do not involve chromosome 11 by cytogenetic detection means.[47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65 and 66] A second nonrandom translocation (Table 33-1), der(16)t(1;16), has been described in a subset of typical Ewing's sarcomas and PPNETs that also possess characteristic tumor-defining translocations. [67,68,69,70 and 71]

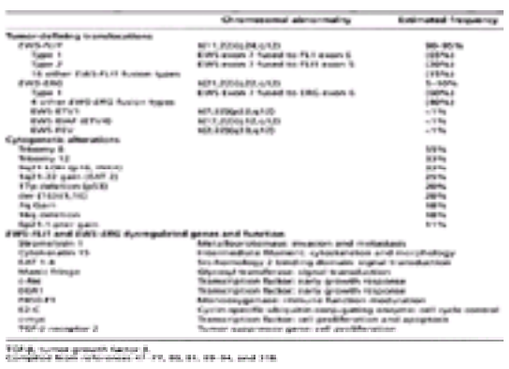


**TABLE 33-1. CYTOGENETIC AND MOLECULAR CHARACTERISTICS OF EWING'S SARCOMA FAMILY OF TUMORS**

Other chromosomal abnormalities include trisomy of chromosomes 8 and 12 and deletion of chromosomes 22, 16q, and 1p36. [51,68,69,70,71 and 72] The ESFT have nonrandom gains, losses, deletions, and loss of heterozygosity at specific chromosomal loci ( Table 33-1), which result in dysfunction of tumor suppressor genes, cell cycle regulators, signal transduction pathways, and yet-to-be-identified genes. In fact, trisomy 8 and 12, loss of 16q and 1p36, and gain of 1q21-22 are linked to distant disease development, relapse, disease progression, and an unfavorable outcome. Consistent nonrandom translocations (EWS-FLI1 and EWS-ERG) in the vast majority (more than 95%) of tumors and nonrandom cytogenetic alterations support a common histogenesis in the ESFT and provide unique diagnostic characteristics to discriminate these tumors from other childhood small round-cell tumors.

Molecular cloning of the breakpoints of the t(11;22)(q24;q12) translocation has localized the rearrangement sites to the EWSR1 region on chromosome 22 and the EWSR2 region on chromosome 11 (Fig. 33-1).[47,48,49,50,51,52,53,54 and 55,73,74,75,76,77,78,79,80 and 81] The EWSR1 region is nested within the novel gene *EWS* on chromosome 22. This gene produces a protein that has homology with other proteins, such as polymerase II, that interact with double-stranded DNA. The EWSR2 region on chromosome 11 contains a subfamily member of the *ets* oncogene superfamily that has 97% homology with the murine friend leukemia integration-1 locus (FLI1).

FLI1 proteins bind to DNA in a sequence-specific manner via the 3'-ets domain and function as a transcriptional activator via the 5'-ets domain. The t(11;22)(q24;q12) chromosomal translocation present in the majority of the ESFT results in rearrangement of the *FLI1* gene with fusion of the carboxy-terminal region of FLI1 with the amino-terminal region of EWS. Because the EWS domain is substituted for a portion of the FLI1 transcriptional domain, the EWS-FLI1 fusion alters the transcriptional activation property usually associated with FLI1. The EWS-FLI1 fusion results in a chimeric protein, which is capable of transforming NIH3T3 fibroblasts in cell culture. Deletion analysis indicates that the EWS segment in the *EWS-FLI1* gene functions as the regulatory or modulating domain. Although the chimeric gene product of the EWS-FLI1 fusion is capable of transforming cell lines, neither EWS nor FLI1 gene products alone have this capability. In the subgroup of the ESFT having the t(21;22)(q22;q12) translocation (Fig. 33-1), EWS from chromosome 22 fuses with another subfamily member of the *ets* oncogene superfamily, ERG, located on chromosome 21.[55,56,81,82] Both ERG and FLI1 are members of the same *ets* oncogene subfamily and have a high degree of homology. The chimeric protein derived from the EWS-ERG fusion is fairly similar to that for the EWS-FLI1 gene product, with EWS replacing the transcriptional domain of ERG. At least 18 EWS-FLI1 and 5 EWS-ERG transcript types have been identified on the basis of the exact fusion exon sites between EWS and the *ets* subfamily member. Despite these differences in transcript types, these chimeric fusion products are capable of transforming cell lines equally well and result in dysregulation of many genes associated with cell signaling, cell proliferation and growth, apoptosis, tissue invasion, and tumor metastasis (Table 33-1).

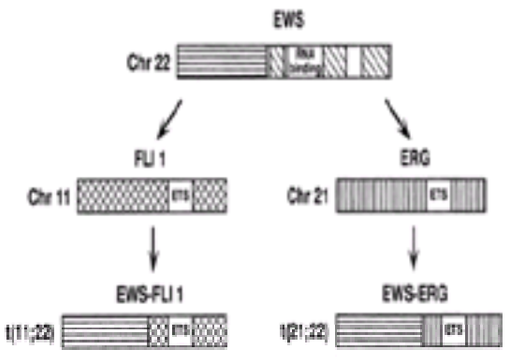



**FIGURE 33-1.** Ewing's sarcoma family of tumors and tumor-defining t(11;22) and t(21;22) translocations resulting in *EWS-FLI1* and *EWS-ERG* gene fusion.

Less common translocations in the ESFT (Table 33-1) have been identified, and the resulting chimeric proteins possess transforming abilities.[51,52,53,54,55] and [56,58] EWS-ETV1 [t(7;22)(p22;q12)] results from fusion of EWS exon 7 with ETV1, which has homology with both FL1 and ERG and is a member of the *ets* oncogene superfamily. Fusion of EWS exon 7 with the adenovirus E1A enhancer-binding protein creates the EWS-EIAF [t(17;22)(p22;q12) translocation. EIAF activates metalloproteinase genes, including stromelysin 1, associated with tissue invasion and tumor metastasis. FEV fuses with EWS exon 7 to create the fifth *ets* superfamily oncogene that results in a tumor-defining translocation [t(2;22)(q33;q12)] in Ewing's sarcoma. Under normal cell conditions, FEV acts as a transcriptional repressor in the adult prostate and small bowel. These three lesser-known Ewing's sarcoma translocations are very infrequent; however, when identified, they represent tumor-defining genetic abnormalities.

Because hybrid transcripts from the fusion of EWS-FLI1 and EWS-ERG have been defined, it is possible to determine the presence or absence of these products in tumor cells. Two molecular genetic techniques commonly are employed. Reverse transcriptase-polymerase chain reaction (RT-PCR) and fluorescent *in situ* hybridization (FISH) may be used to detect the specific EWS-FLI1 or EWS-ERG hybrid transcript expressed by the tumor.[82,83,84,85,86,87] and [88] RT-PCR depends on reverse transcription of RNA from tumors into complementary DNA and amplification of the chimeric transcripts to detect the gene products. Using primers derived from the genes participating in the chromosomal translocation carries out amplification of only the specific fusion sequence. Such a technique has been shown to be fairly specific and highly sensitive in detecting hybrid transcripts.[60,82,83,84,85] and [86] In fact, a hybrid transcript for either EWS-FLI1 or EWS-ERG may be identified in more than 95% of typical or atypical Ewing's sarcomas and PPNET tumors.[51,52,53,54,55,56,57,58,59] and [60] Within one particular study, hybrid transcripts were identified by RT-PCR in 40% of undifferentiated tumors, allowing for reclassification of these tumors into the ESFT. A high correlation level of RT-PCR detection of hybrid transcripts with cytogenetic findings is present for both typical t(11;22)(q24;q12) or t(21;22)(q22;q12) and complex or variant translocations, with EWS-FLI1 or EWS-ERG fusion transcripts identified in 98% of cases. Perhaps even more intriguing is the identification of EWS-FLI1 or EWS-ERG products in 75% of the ESFT with no cytogenetic evidence of structural rearrangement for chromosomes 11, 21, or 22. Similarly, hybrid transcripts may be localized and identified by FISH with a similar degree of sensitivity and specificity.[86,87] and [88] With FISH, neoplastic cells are hybridized directly with probes for the two involved loci, EWS-FLI1 and EWS-ERG. Gene fusion is evaluated by direct visualization of fluorescent labeling in interphase cells. Translocation has occurred if the bivariate hybridization labels are adjacent to each other on the same chromosome.

With the ability to determine the precise EWS-FLI1 and EWS-ERG fusion transcript type, the distribution of transcript types within Ewing's sarcoma tumors has been determined (Table 33-1).[51,52,53,54,55] and [56,58,89,90,91,92,93] and [94] The clonality of Ewing's sarcoma is emphasized by the fact that only a single specific type of fusion transcript is identified in an individual tumor. This specificity applies to both primary and metastatic tumors in the same patient and in tumor at relapse. Type 1 and type 2 transcripts occur in most (85%) of EWS-FLI1 Ewing's sarcomas, whereas EWS-ERG type 1 fusion transcript accounts for 60% of EWS-ERG tumors, with four non–type 1 transcripts comprising the remaining 40%. More importantly, clinicopathologic studies indicate that EWS-FLI1 type 1 transcript (EWS exon 7 fusion with FLI1 exon 6) is associated independently with stage of disease at diagnosis, improved prognosis, increased overall and disease-free survival (DFS), relapse, and reduced metastatic potential, when compared with non–type 1 EWS-FLI1 transcripts.[89] Median survivals for either all patients or those with only localized disease were identical: 27 months for non–type 1 transcripts and 113 months for type 1 transcript. The risk ratio for developing metastatic disease was reduced by almost threefold in those with type 1 fusion transcripts. The reason that type 1 EWS-FLI1 transcripts provide a more favorable clinical course is not known with certainty. Native FLI1 exons 4 to 8 contribute to transactivation, and exons 5 to 8 modulate protein-protein interactions with a serum response factor. Structural features of FLI1 are controlled by exon 5 (helical domain) and exons 6 and 7 (predicted turn). The absence of exon 6 may affect the spatial relationship of the chimeric EWS-FLI1 protein's DNA-binding domain with the corresponding target's transactivation domain. Ewing's sarcoma and NIH3T3 cell culture investigations have shown a less active chimeric transcription factor, with type 1 EWS-FLI1 transcript and reduced transactivation of FLI1-responsive reporter constructs. This implies that exon 6 may have a modulating or inhibitory effect on transactivation with EWS-FLI1 type 1 and that this may be reflected as a more favorable clinical course. At present, treatment decisions have not been based on specific EWS-FLI1 transcript types; however, fusion transcript types may play a role in future therapy.

EWS-ERG fusion type analysis has identified at least five distinct transcripts.[51,54,55,92] As noted, 60% are type 1 EWS-ERG transcripts (EWS exon 7 fused with ERG exon 6). The number of tumors with the EWS-ERG translocation is fairly small in comparison with those having the EWS-FLI1 translocation. Differentiation between EWS-ERG fusion types with respect to clinical features will require accumulation of an adequate number of cases to provide statistically meaningful data. However, comparison of clinical factors between EWS-FL1 and EWS-ERG fusion transcripts found no differences in age at diagnosis, gender, primary tumor site, metastatic disease at diagnosis, relapse rates, event-free survival (EFS), or overall survival. Similarly, EWS-FL1 type 1 and EWS-ERG type 1 fusion transcripts had similar clinical phenotypes. EWS-FLI1 and EWS-ERG appear to result in similar clinical presentations and outcomes.[92] This finding implies that the transcripts from EWS-FLI1 and EWS-ERG have comparable functions in tumorigenesis.

RT-PCR and FISH for EWS-FLI1 and EWS-ERG fusion transcripts may provide the diagnostic information necessary to confirm the histopathologic suspicion of Ewing's sarcoma in particularly difficult cases.[51,52,53,54] and [55,59,60] The benefit of both RT-PCR and FISH is that the detection of a t(11;22) or t(21;22) translocation may be performed in a considerably shortened period when compared with cytogenetic culture means. In addition, certain tumors without chromosome 11, 21, or 22 abnormalities may express hybrid transcripts that are detected only by RT-PCR, FISH, or other molecular techniques. Aspiration or fine-needle biopsies of tumors may be evaluated readily by RT-PCR and FISH to confirm the diagnosis of the ESFT.[59,95,96] and [97] This is especially useful when limited tissue is available for diagnosis or cytogenetic cultures fail to reveal tumor-defining translocations.

Peripheral blood and bone marrow evaluation for the presence of Ewing's sarcoma cells is possible with RT-PCR for EWS-FLI1 and EWS-ERG fusion products.[98,99,100] and [101] Some data are controversial regarding the utility of these studies in predicting relapse, metastatic disease, and outcome. Circulating Ewing's sarcoma cells in peripheral blood may be detected in 30% of patients with localized disease and in 50% of patients with metastatic/relapsed disease.[99,100] Likewise, Ewing's sarcoma cells identified by RT-PCR have been found in 19% of patients with localized disease and in 33% of patients with metastatic/relapsed disease. Peripheral blood or bone marrow (or both) was positive in 25% of patients with localized disease as compared with 50% for patients with metastatic/relapsed disease identified by RT-PCR. Overall survival at 18 months was not statistically different between those with RT-PCR–negative and RT-PCR–positive peripheral blood.[99] In

contrast, RT-PCR–positive bone marrow resulted in a significant reduction in overall survival over a limited 12-month period. [99] Another study showed that all individuals with RT-PCR–positive peripheral blood and bone marrow test results became negative after chemotherapy. [101] After a median follow-up of 30 months, relapses were observed in three of seven patients with RT-PCR–positive bone marrow test results, as compared with 2 of 16 patients with RT-PCR–negative bone marrow test results. In this 30-month study, bone marrow involvement was determined not to allow prediction of early relapse. These studies do highlight the fact that in a substantial portion of localized disease cases, Ewing's sarcoma is a systemic disease with circulating tumor cells and reinforces the need for systemic chemotherapy in controlling and eradicating the neoplastic cells. [98,99,100 and 101] Further studies of a more longitudinal nature are needed to determine the significance of RT-PCR–positive peripheral blood and bone marrow.

Timing of peripheral blood collection in RT-PCR studies is important. Ewing's sarcoma cells have been identified in peripheral blood immediately after biopsy. [98] No such cells were detected by RT-PCR before or 6 days after tumor biopsy. Perhaps, peripheral blood or bone marrow RT-PCR for EWS-FLI1 and EWS-ERG transcripts may be more appropriate for postchemotherapy evaluation of patients with no evidence of disease or with stable disease. This may allow for early detection of relapse or minimal residual disease.

Use of molecular techniques has defined a biphenotypic sarcoma of possible neural crest origin that expresses both the tumor-defining cytogenetic translocation [t(11;22)(q24;q12)] and the EWS-FLI1 fusion transcript characteristic for the ESFT. [102] These particular tumors were classified previously as embryonal and alveolar rhabdomyosarcomas on the basis of morphologic, immunocytochemical, and ultrastructural features. In addition, these tumors possessed neural differentiation, as shown by immunoreactivity for neurofilaments and the presence of primitive neurites and neurosecretory dense core granules by electron microscopy. By RT-PCR and Northern analyses, neural-associated gene expression for choline acetyltransferase, chromogranin A, neuron-specific enolase (NSE), and human neurofilaments intimately linked with the ESFT were identified. Myogenic regulatory genes ( *MYF5*, myogenin, and *MYOD1*) were assayed by Northern analysis. These biphenotypic tumors expressed *MYF5* and myogenin, but *MYOD1*, which characteristically is present in rhabdomyosarcoma, was not found with these tumors. Also, *MYOD1* expression in cell lines from these biphenotypic tumors using media known to promote myogenic differentiation could not be induced. The implication from this multimodal investigation is that the EWS-FLI1 oncoprotein may inhibit *MYOD1* expression while promoting neural differentiation as evidenced in the ESFT. Similar suppression of *MYOD1* expression has been found with other oncogenes and proto-oncogenes, in particular *Ras* and c-*fos*.[103] These biphenotypic tumors potentially represent “primitive” malignant ectomesenchymomas related to the ESFT.[102] Typical malignant ectomesenchymoma tumors are neural crest–derived, are composed of neoplastic cells with neural differentiation, and contain one or more malignant mesenchymal components, often including rhabdomyosarcoma. At least 10% of primitive sarcomas with myogenic differentiation have been estimated to be biphenotypic sarcomas with *EWS-FLI1* gene fusion.[102,104] These biphenotypic sarcomas appear to be related to the ESFT. In addition to biphenotypic sarcomas, other small round-cell tumors may represent variants within the ESFT. Certain polyphenotypic tumors, rare mixed alveolar and embryonal rhabdomyosarcomas, malignant ectomesenchymomas, and olfactory neuroblastomas (esthesioneuroblastoma) possess the *EWS-FLI1* translocation.[105,106] Diagnoses in these particular cases must be predicated on histopathologic, immunocytochemical, and genotypic features. These recent revelations emphasize the utility of molecular genetics in conjunction with morphologic analyses in the classification and understanding of the pathobiology of solid childhood tumors.

Although certain tumors are associated with overexpression of mutated forms of tumor suppressor genes, such as mutated *Rb* or *p53*, relatively few tumors and cell lines within the ESFT have been evaluated. Molecular studies have shown that although mutated *p53* expression is relatively common in Ewing's sarcoma tumor cell lines, it is a rare event in primary tumors. [107,108] It was surmised that tumor cells in cell cultures with a mutated *p53* gene (chromosome 17p13) might dominate over cells with an intact wild-type *p53* gene. This theory is corroborated also by the failure to show a significant number of primary tumors with mutated *p53* gene product expression by PCR. The most likely explanation for the increased expression of mutated *p53* in cell cultures is that these mutations are acquired at a later stage in tumorigenesis and may be involved in progression of an already established tumor. Additional data regarding this well-known tumor suppressor gene and other tumor suppressor genes and their role in the ESFT undoubtedly will be forthcoming in the near future.

Finally, the neoplasms composing the ESFT are distinct from neuroblastoma with respect to N-*myc* expression.[42,43 and 44] Molecular studies have shown that the ESFT do not exhibit N-*myc* amplification and also lack high levels of c-*src* tyrosine kinase activity and c-*cts*-1 oncogene expression.[42,43 and 44,109] In contrast, the ESFT express relatively high levels of c-*myc*, which are not found in neuroblastoma. Other small round-cell tumors of childhood, such as rhabdomyosarcoma and lymphoma, possess increased expression of c-*myc* as well.[109,110,111 and 112] N-*myc* and c-*myc* are oncogenes that are amplified in neoplastic processes and participate in transformation, proliferation and may have prognostic significance. N-*myc* is associated classically with neuroblastoma, located on chromosome 2 (2p24) and, when amplified, is associated with a poor prognosis. C-*myc* is found with several neoplastic processes, including leukemias, lymphomas, and carcinomas of the lung, esophagus, cervix, oral mucosa, and ovary. This oncogene is located on chromosome 8 (8q22). In pediatric neoplasia, c-*myc* is found in Ewing's sarcoma and not in neuroblastoma and may be helpful in differentiating between these neoplasms because neuroblastoma expresses N-*myc*. Likewise, the *dbl* oncogene has been detected in both the ESFT and neuroblastoma.[28,29 and 30,33,113] Although lack of N-*myc* amplification in Ewing's sarcoma may be helpful in differentiating neuroblastoma from this tumor, it is helpful only when N-*myc* amplification is present with an undifferentiated tumor. The absence of N-*myc* amplification is not useful in an undifferentiated neoplasm, which lacks morphologic, immunocytochemical, and ultrastructural features of Ewing's sarcoma. In such cases, the role of cytogenetics and molecular genetics in providing a definitive diagnosis is paramount.

The application of molecular genetics, in particular RT-PCR and FISH, renders possible the evaluation of tumors that in the past failed to establish the cell growth necessary for typical cytogenetic studies. With rapid advancement in molecular techniques, such instruments will play a prominent role in diagnosis, follow-up, and evaluation of residual, recurrent, and metastatic disease in childhood tumors.

## DNA PLOIDY AND PROLIFERATIVE INDICES

A paucity of studies has analyzed DNA indices and proliferative indices in the ESFT. [114,115,116,117,118,119 and 120] The majority of studies include only a few cases along with other bone tumors and soft tissue sarcomas. Comparison of DNA content by cytophotometric and flow cytometry in a retrospective study has provided interesting results in 37 patients with Ewing's sarcoma of bone.[114] Diploid tumors were identified by cytophotometry and flow cytometry in 67% and 69% of cases, respectively. Of diploid cases, 58% of individuals in the cytophotometry group and 53% in the flow cytometry group were alive 7.5 years and 8.2 years after diagnosis, respectively. All patients with aneuploid tumors died of disease, with a mean survival of 32 months in those in the cytophotometry group and 35 months in those in the flow cytometry group. A relatively small number (n = 13) of PPNET and EES were included in a separate flow-cytometric study [116] of childhood soft tissue sarcomas. Slightly more than 75% of tumors were diploid or near-diploid (DNA index, 1.00 to 1.09), with 23% of tumors being aneuploid. The median S-phase value was 9.7%. Survival statistics and comparison with DNA data were not performed for PPNET and EES.

Using proliferating cell nuclear antigen (PCNA) and Ki-67 (MIB1) antibody, a clinicopathologic study [115] evaluated proliferation-associated markers in Ewing's sarcomas of bone in a pediatric population. PCNA/cyclin is a nonhistone nuclear protein that functions as a cofactor with DNA polymerase delta. [121] This protein may be detected in cells within the proliferating phases of the cell cycle ($G_1$ through $G_2$/M), with the highest level of detection from mid-$G_1$ through S phases. Quiescent cells ($G_0$) do not express this protein. The monoclonal antibody Ki-67 detects an antigenic epitope of a nuclear protein associated with cell proliferation. This protein may be detected from mid-$G_1$ through $G_2$/M.[121] Noncycling ($G_0$) and early $G_1$ phase cells are nonreactive with the Ki-67 antibody. The tumors showed a relatively high level of expression of both PCNA and Ki-67 detectable proteins by immunocytochemical techniques. [115] Elevated PCNA and Ki-67 proliferation marker expression and survival were associated, in that statistically significant differences were noted between a group that included individuals who either died of disease or were alive with disease, and a group that showed no evidence of disease. Individuals with higher proliferative indices were more likely to die of disease or be alive with disease, and those with lower proliferative indices were more likely to have no evidence of disease. This finding implies that proliferation markers or the proportion of neoplastic cells in the S+$G_2$/M phase of the cell cycle (or both) should be assessed and may provide some insight into the aggressiveness and prognosis in Ewing's sarcoma.

As discussed, EWS-FLI1 type I fusion is associated with improved prognosis. Evaluation of Ki-67 expression in EWS-FLI1 and EWS-ERG was compared with special reference to EWS-FLI1 type 1 tumors. Ki-67 expression was increased significantly in non–type 1 EWS-FLI1 type 1 tumors (24%) as compared with both EWS-FLI1 type 1 (15%) and EWS-ERG (35%) Ewing's sarcomas.[122] Categorical evaluation of all tumors with low (less than 15%) and high (more than 15%) expression of this proliferation marker indicated that 67% of EWS-FLI1 type 1 fusions had low expression, whereas 60% of type 2 EWS-FLI1 and 79% of EWS-ERG tumors had high Ki-67 expression.

Of particular interest was the significant correlation between Ki-67 and IGF-1 receptor immunoreactivity with EWS-FLI1 type 1 tumors (IGF-1R, 65%; Ki-67, 15%) as compared with non–type 1 EWS-FLI1 tumors (IGF-1R, 82%; Ki-67, 24%).[122] With all Ewing's sarcomas, IGF-1R was expressed in more than 50% of tumor cells when Ki-67 was detected in more than 20% of tumor cells. When less than 10% of tumor cells expressed Ki-67, IGF-1R was detected in fewer than 40% of tumor cells. *In situ* DNA nick end labeling analysis for apoptosis in EWS-FLI1 and EWS-ERG found a trend toward increased apoptosis in EWS-ERG (10.1%) as compared with

EWS-FLI1 type 1 (2.4%) and non–type 1 tumors (5.3%).[122] The overexpression of proliferation markers in non–type 1 EWS-FLI1 and EWS-ERG tumors would appear to be mediated by an IGF-1R pathway. IGF-1 and IGF-1R are known to participate in the neoplastic process by autocrine and paracrine mechanisms. However, other regulators of the cell cycle also may contribute to an increase in Ki-67 protein production, such as mutated *p53* (11% of tumors) and INK4A (17% of tumors).[122] Overexpression of both Ki-67 and IGF-1R is associated with tumor progression and portends a poor prognosis. EWS-FLI and EWS-ERG chimeric proteins may modulate expression of the *IGF-1R* gene. Inhibition of *IGF-1R* may lead to reduced proliferation and increased apoptosis in Ewing's sarcoma.

In addition to PCNA and Ki-67, other proliferation markers[121] are available and may become important in evaluating the ESFT. A thymidine analog 5-bromodeoxyuridine (BrdU) requires incubation with fresh viable tissue and is taken up by synthetically active (S phase) cells. The incorporation of BrdU may be demonstrated by immunocytochemistry, immunofluorescence, and flow cytometry. This monoclonal antibody has, to a great extent, replaced tritiated thymidine as a proliferation marker. DNA polymerase alpha is expressed in proliferating cells from $G_1$ through $G_2$/M and is a cell cycle–related enzyme involved with DNA synthesis. This proliferation marker has a sensitivity that is two to three times less than that for PCNA and requires frozen tissue for analysis. Monoclonal antibody p105 is directed against two proliferation-associated nuclear proteins of 105 kD and 41 kD. This flow-cytometric and immunocytochemical antibody reacts with a nuclear antigenic epitope within chromatin granules of the nuclear matrix involved with RNA synthesis. The detected protein p105 may modulate regulatory functions involved with terminal RNA transcript production required for progression in the cell cycle. This protein is expressed in all proliferating cell phases ($G_1$ through $G_2$/M) but has its greatest expression in late S and M phases. Immunoreactivity to p105 and Ki-67 has been shown to correlate well. This antigen withstands formalin fixation and paraffin embedding of the tissue. However, optimal use of this monoclonal antibody has been with bivariate flow cytometry.

DNA flow cytometry and proliferation markers, such as Ki-67 (MIB1), PCNA, and BrdU, have been employed in numerous clinicopathologic studies and have predictive value. Clinical stage, histologic grade, tumor recurrence, and survival have been linked to increased expression of these monoclonal antibodies and both DNA ploidy (aneuploidy) and elevated proliferative fraction (S+$G_2$/M) status within a variety of tumors.[121] At present, relatively few clinicopathologic studies have been performed with the ESFT,[114,116,117,118,119 and 120] and the role of DNA ploidy and proliferative index markers is in the process of being defined.

## PROSPECTIVE INNOVATIVE BIOLOGY-BASED THERAPY

With the identification of EWS-FLI1 and EWS-ERG chimeric proteins' transforming ability in Ewing's sarcoma, creation of antisense oligodeoxynucleotides to these proteins allows for prevention of messenger RNA (mRNA) translation. *In vitro* studies with Ewing's sarcoma cell lines expressing EWS-FLI1 have found a 40% to 60% reduction in fusion protein production in the presence of antisense material directed toward EWS-FLI1.[123,124 and 125] In addition, tumor cell growth was inhibited by 25% to 40%. The ability to transfect Ewing's sarcoma cell lines with EWS-FLI1 and EWS-ERG antisense using plasmids has shown encouraging results. Previously tumorigenic cell lines after transfection have proved to be nontumorigenic in an athymic murine model. These transfected cell lines also have impaired colony formation, anchorage-independent growth on soft agar, and a ten- to 20-fold decrease in clonogenic efficiency. Along with these phenotypic changes, a significant loss of EWS-FLI1 and EWS-ERG fusion protein expression occurred. Other alternatives to antisense in down-regulating the transforming EWS fusion products include ribozymes, neutralizing antibodies, truncated *ets* oncogene domain–binding molecules, and novel drugs.

Several cell-surface receptors that participate in signal transduction and cell growth are expressed in Ewing's sarcoma and also may be therapeutic targets.[38,39,40 and 41,126,127 and 128] Ewing's sarcoma cell lines and tumors display receptors for IGF-1, gastrin-releasing peptide, transforming growth factor-b type II, CD40, and Fas.[38,39,40 and 41,126,127 and 128] These neoplastic cells also interact with G protein–coupled receptors that regulate signal transduction and cell growth.[126] This particular receptor is modulated by a protein tyrosine kinase (Pyk2) that regulates the interaction of RNA-binding protein EWS with G protein–coupled receptors. Treatment of Ewing's sarcoma with ligands to these various receptors may derail the signal transduction effect of EWS fusion proteins. Apoptosis also may be induced via the Fas receptor expressed on tumor cell surfaces.[128] Several Ewing's sarcoma cell lines possess Fas ligand that is faulty and is incapable of inducing apoptosis in cytotoxic lymphocytes (Jurkat cells). Ewing's sarcoma cell lines serve as stimulators for generation of cytotoxic effector lymphocytes and are susceptible to lysis. Immunotherapy would appear possibly to be effective in treatment of Ewing's sarcoma. With the numerous receptors and growth factors interacting with EWS fusion proteins, the need for characterization of individual tumors may be necessary in the near future to direct biologically based therapy.

## PATHOLOGY

### Classification

The ESFT represent a spectrum from undifferentiated Ewing's sarcoma to poorly differentiated atypical Ewing's sarcoma to differentiated PPNET. Differentiation in this family of tumors is based on expression of morphologic, immunocytochemical, ultrastructural, and molecular neural features. The term *PPNET* includes soft tissue and bone neoplasms previously termed *peripheral neuroepithelioma*, *adult neuroblastoma*, and *malignant small-cell tumor of the thoracopulmonary region (Askin tumor)*, which is associated with the chest wall and thoracic cavity. The ESFT include both osseous and extraosseous forms. Before sophisticated cytogenetic and molecular techniques, a number of undifferentiated tumors were included in EES. With the currently available diagnostic instruments, tumors within this category are less likely to include neoplasms that do not meet the criteria for the ESFT. As noted, such additional tumors as the biphenotypic sarcoma may be related to the ESFT and could in the future represent a distinct subtype, especially if clinical behavior and response to oncologic management are similar to those for the ESFT.

### Triaging Tissue and Processing of Biopsy and Resection Specimens

At initial tumor biopsy, a pathologist must assess the quantity of viable tumor tissue submitted to determine whether it is adequate for diagnosis and various studies. Tissue should be submitted for (a) cytogenetic (karyotype) studies in tissue culture media; (b) molecular, RT-PCR, and specialized immunocytochemical studies (cryopreserved at –70°C); (c) flow-cytometric studies for DNA ploidy and proliferation fraction in tissue culture media; (d) ultrastructural examination in glutaraldehyde; (e) light-microscopical examination and routine immunocytochemical and FISH studies in 10% buffered formalin and alcohol; and (f) cytologic imprints (alcohol-fixed and air-dried) for FISH studies. If a needle biopsy is performed, multiple tissue cores are necessary to allow for cytogenetic, molecular, routine, and ultrastructural studies.[59,120] In a cytologic study of 20 extraskeletal Ewing's sarcomas, diagnostic tumor material for routine light-microscopical and immunocytochemical studies was available in all cases.[97] EWS-FLI1 translocations were identified within the ten tumors from which tissue was available for cytogenetic and molecular evaluation.

Surgical resection of the tumor after chemotherapy induction requires that soft tissue, bone marrow, and bone surgical margins be assessed thoroughly to determine negative surgical margins and the distance of the tumor to the closest surgical margin. The attached skin and soft tissue containing the original biopsy site is sampled thoroughly for residual tumor. If tumorous tissue is readily accessible, tumor tissue should be submitted for cytogenetic and molecular analysis, especially if they were not performed on the initial biopsy or if prior studies failed to identify a tumor-defining translocation. Cytogenetics may be informative regarding additional chromosomal abnormalities associated with treatment effect, progressive disease, and prognostic implications. Likewise, tissue for DNA ploidy and proliferation fraction may be informative if not completed previously. Before sectioning, the specimen should undergo radiographic examination in at least two planes to determine residual tumor location and extent. The entire specimen is frozen at –70°C for several hours. The frozen specimen then is sectioned serially in a longitudinal direction, allowing for exposure of the maximum dimension of the tumor. The tumor size is measured in three dimensions. The entire longitudinal section containing the maximum tumor area is mapped diagrammatically, is photographed, and is submitted in multiple blocks for evaluation of the histologic response to therapy. All areas of this longitudinal section, including the medullary canal contents, residual tumor, cortical bone, surgical margin, and immediately surrounding soft tissue, are fixed in buffered formalin. The calcified tissue blocks undergo decalcification with a formic acid-formalin solution before processing. The degree of decalcification may be determined by radiographic evaluation of the blocks. Other selected areas of interest from the remaining longitudinal sections of the specimen are submitted for evaluation also.

### Gross Appearance

Both soft tissue and osseous ESFT have a similar appearance (Fig. 33-2). Even with a primary tumor that originates intraosseously, a soft tissue component may be present owing to extension through the cortical plate. Likewise, some tumors may arise from soft tissue and invade the adjacent bone. These neoplasms are composed of firm, gray-white soft tissue with a glistening, moist appearance on sectioning. Extraosseous tumors tend to have a less firm texture and are more friable. Hemorrhage and cystic degeneration secondary to tumor necrosis are a common feature as well. With intraosseous neoplasms, the medullary cavity often is involved diffusely, and the extent of tumor involvement is considerably greater than that appreciated on conventional radiographs.

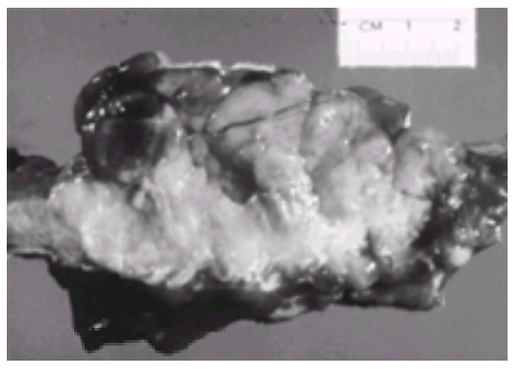


**FIGURE 33-2.** Typical gross appearance of Ewing's sarcoma family of tumors.

### Histopathologic Features

The histopathologic features of the ESFT, as determined by light microscopy, are summarized in Table 33-2.

| Feature | Ewing's sarcoma | | PPNET |
|---|---|---|---|
| | Typical | Atypical | |
| Light microscopy | | | |
| Pattern | Sheets of cells | Usually nesting | Alveolar, rosettes, organoid, or lobular pattern |
| Intercellular matrix | Absent | Usually present | Present |
| Cell size | Small | Small to large | Small to large |
| Cell shape | Exclusively round | Predominantly round | Predominantly round |
| Cytoplasm | Scant | Scant to easily identifiable | Easily identifiable |
| Nucleus | Round/smooth | Round, occasionally oval | Round, occasionally oval |
| Mitoses | <2 per high-power field | <2 per high-power field | <2 per high-power field |
| Electron microscopy | | | |
| Cell shape | Round/smooth | Round to oval, irregular | Round to oval, irregular, cell processes |
| Nuclear shape | Round | Round to oval | Round to oval |
| Neurosecretory granules | Absent | Absent | Present |
| Organelles | Sparse | Moderate | Moderate to many |
| Filaments | Absent | Absent to few | May be present |
| Glycogen | Present | Present | Present |
| Attachments | Primitive | Primitive to moderately developed | Moderately to well developed |
| Basal lamina | Absent | Absent | Absent |
| Extracellular matrix | Absent | Variable | Variable |

PPNET, peripheral primitive neuroectodermal tumor

**TABLE 33-2. EWING'S SARCOMA FAMILY OF TUMORS: HISTOPATHOLOGIC AND ULTRASTRUCTURAL FEATURES**

#### *Typical Undifferentiated and Atypical Poorly Differentiated Ewing's Sarcoma*

As initially described by James Ewing[1] in 1921, typical undifferentiated Ewing's sarcoma is composed of broad sheets of polyhedral cells with small hyperchromatic nuclei, relatively well-defined cell borders, amphophilic cytoplasm, a high nuclear-cytoplasm ratio, and absence of intercellular material ( Fig. 33-3). Additional cytologic features include a monomorphous appearance of the tumor cells, scant cytoplasm, homogenous nuclear basophilia with inconspicuous nucleoli, and rare mitotic figures (fewer than two mitoses per high-power field).[45,59,120,129,130,131,132,133,134,135] and [136] The cells are in close proximity to each other with no detectable intervening stromal supporting elements, even with reticulin staining. The broad sheets of tumor cells occasionally are interrupted by delicate fibrovascular septa containing small-caliber vessels and capillaries. Usually, considerable areas of hemorrhage and associated tumor necrosis are present. The amphophilic or clear nature of the cytoplasm is due to the abundance of glycogen.[59,120,129,130,131] and [132] The presence of glycogen may be demonstrated by periodic acid–Schiff (PAS) staining without diastase digestion. Although most tumor cells contain intracytoplasmic glycogen, formalin fixation may result in suboptimal fixation of glycogen and loss of PAS positivity for glycogen. Preservation of glycogen is accomplished by alcohol-based fixation. Although many individuals equate abundant cytoplasmic glycogen in a small blue-cell tumor of childhood with Ewing's sarcoma, this finding alone is not sufficient for the diagnosis and cannot differentiate this tumor from other childhood small blue-cell tumors. The tumor typically invades the adjacent normal tissue by compression, giving the tumor a “pushing” type of margin. An infiltrative pattern also may be seen but more commonly is seen with atypical poorly differentiated Ewing's sarcoma.

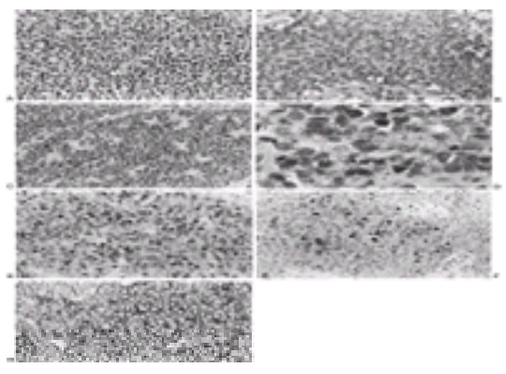


**FIGURE 33-3.** Histopathologic and immunocytochemical appearance of Ewing's sarcoma family of tumors. **A:** Typical undifferentiated Ewing's sarcoma. **B:** Atypical poorly differentiated Ewing's sarcoma. **C:** Peripheral primitive neuroectodermal tumor (differentiated Ewing's sarcoma). **D:** Detection of glycogen in typical undifferentiated Ewing's sarcoma (periodic acid–Schiff histochemistry). **E:** Proliferating cell nuclear antigen (PCNA) expression in typical Ewing's sarcoma from patient who died of disease (PCNA immunocytochemistry). **F:** PCNA expression in typical Ewing's sarcoma from patient who has no evidence of disease at follow-up (PCNA immunocytochemistry). **G:** Diffuse cell membrane immunoreactivity for glycoprotein p30/32$^{MIC2}$ in Ewing's sarcoma family of tumors (HBA-71 immunocytochemistry).

Atypical poorly differentiated Ewing's sarcoma (Fig. 33-3B) is characterized by an increase in overall cell and nuclear size, cellular pleomorphism with ovoid cell shape, and irregular nuclear contours and an increased mitotic rate (greater than two mitoses per high-power field). [45,59,120,129,130,131,132,133,134,135] and [136] The nuclei show a dispersed chromatin appearance with relatively prominent nucleoli. Rare or complete absence of glycogen on PAS staining is characteristic. Atypical Ewing's sarcoma may be arranged in sheets, or have an organoid, lobular, or alveolar architectural pattern. The presence of an organoid, lobular, or alveolar pattern qualifies the tumor as an atypical poorly differentiated form even if cytologic features resemble typical undifferentiated Ewing's sarcoma. Detectable intervening eosinophilic supporting stroma are seen between groups or individual tumor cells. Focal tumor necrosis and hemorrhage also may be seen. Spindle cells may be present at the periphery of the tumor, and these tumor cells invade the adjacent tissue in an infiltrative pattern. Both typical (undifferentiated) and atypical (poorly differentiated) Ewing's sarcoma lack morphologic features of neural differentiation, such as pseudorosettes, true rosettes, or a neuropil background.

EES may be classified using the same criteria but more commonly has an organoid, alveolar, lobular, or even a pericytic architectural pattern. [45,120,129,130,131,132,133,134,135] and [136] Differentiation from other soft tissue masses and small blue-cell tumors of childhood are dependent on immunocytochemical, ultrastructural, cytogenetic, and molecular genetic findings.

#### *Peripheral Primitive Neuroectodermal Tumor (Differentiated Ewing's Sarcoma)*

The PPNET member of the ESFT may have histopathologic features that resemble typical undifferentiated or atypical poorly differentiated Ewing's sarcoma; however, a neural immunophenotype or neural differentiation on ultrastructural examination will be present. [45,59,120,129,130,131,132,133,134,135] and [136] On the basis of histopathologic

criteria alone, Flexner-type rosettes or Homer Wright–type pseudorosettes may be present in PPNET ( Fig. 33-3C). Flexner rosettes are glandlike structures that are seen infrequently in neural tumors, whereas Homer Wright pseudorosettes are annular arrays of tumor cells surrounding central zones of eosinophilic fibrils. Rarely will true rosettes be identified. More typically, the neoplastic cells are organized into an organoid, alveolar, or lobular pattern ( Fig. 33-3) with infrequent to rare pseudorosettes. This differentiated form of the ESFT lacks mature neural elements, such as ganglion cells, nerve fascicles or bundles, and a neuropil background. The background is composed of a fibrovascular network with eosinophilic supporting stroma. Focal regions of tumor necrosis and hemorrhage also may be seen. Increased mitotic activity is present. Included within this category is the malignant small-cell tumor of the thoracopulmonary region (Askin tumor) that involves the chest wall more commonly in adolescent women and is believed to be derived from pluripotent cells along intracostal nerves.

### Ultrastructural Features

The ultrastructural features of the ESFT, as determined by electron microscopy, are summarized in Table 33-2.

#### *Typical Undifferentiated Ewing's Sarcoma and Atypical Poorly Differentiated Ewing's Sarcoma*

Typical undifferentiated Ewing's sarcoma ( Fig. 33-4A) is composed of small homogenous cells with round to oval nuclei with smooth nuclear contours. [45,59,120,129,130,131 and 132,137,138,139 and 140] The nuclei have dispersed chromatin with relatively little peripherally placed heterochromatin. Occasional nucleoli are seen. The cytoplasm is relatively bland with sparse organelles and only occasional mitochondria, lipid droplets, and polyribosomes. The predominant cytoplasmic feature is relatively abundant glycogen deposition with some areas described as glycogen pools or lakes. Intermediate filaments, neurofilaments, and extracellular matrix are not appreciated. Neurites or cell processes are rare to absent. The tumor cells are in close proximity and have rudimentary cell attachments.

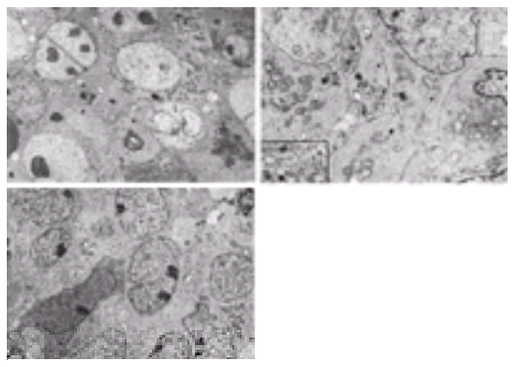


**FIGURE 33-4.** Ultrastructural appearance of Ewing's sarcoma family of tumors. **A:** Typical undifferentiated Ewing's sarcoma composed of round primitive cells with scant cytoplasm containing glycogen. **B:** Peripheral primitive neuroectodermal tumor with prominent neurite-like cell processes containing dense core neurosecretory granules (*inset*). **C:** Atypical poorly differentiated Ewing's sarcoma composed of cells with slightly irregular nuclear contours, decreased cytoplasmic glycogen, and occasional rudimentary cell processes.

Atypical, poorly differentiated Ewing's sarcoma is composed of larger cells with a moderate degree of cellular and nuclear pleomorphism ( Fig. 33-4C).[45,59,120,129,130,131 and 132,137,138,139 and 140] The cell borders are irregular, with areas of cellular molding and intervening cell processes. The cell processes rarely show dense core granules and filaments. An increase in peripherally placed heterochromatin and more prominent nucleoli are present. The cytoplasm contains a reduced amount of glycogen as compared with typical (undifferentiated) Ewing's sarcoma. Rudimentary cell attachments are more frequent and easily recognized. The extracellular space may show occasional areas of collagen deposition. No other tumor-defining characteristics are seen.

#### *Peripheral Primitive Neuroectodermal Tumor (Differentiated Ewing's Sarcoma)*

Intertwining neurite-like processes with dense core granules ( Fig. 33-4B) are seen with PPNET (differentiated Ewing's sarcoma). [45,59,120,129,130,131 and 132,137,138,139 and 140] These cytoplasmic processes are organized in a haphazard manner, and occasional processes will contain neurofilaments and microtubules. The dense core granules are reduced in number and usually exhibit an angulated or pleomorphic morphology as compared with those present in neuroblastomas. The cytoplasm may contain occasional small aggregates of glycogen. The nuclei tend to be ovoid to slightly elongated and are somewhat irregular in contour. Prominent nucleoli and peripherally placed heterochromatin characterize the nuclear morphology. Relatively well-developed cell attachments are seen. Occasional pseudorosettes with central areas composed of intertwining cell processes may be present. Fusiform cells resembling fibroblasts or Schwann cells may occupy the stromal areas, and both typical and long-spacing collagen deposition occasionally is found. No other tumor-defining characteristic is present.

### Immunocytochemistry

Differentiation of the ESFT from other small round-cell tumors of childhood may prove to be difficult on the basis of morphologic features alone. Monoclonal and polyclonal antibodies for various differentiation markers [45,59,120,129,130,131 and 132,135,136,137,138,139,140,141,142,143,144,145,146,147,148 and 149] may provide diagnostic clues. Table 33-3 presents a comparison of immunoreactivity for various markers within the ESFT and among other small blue-cell tumors of childhood. In the past, typical undifferentiated Ewing's sarcoma was defined as a neoplasm that had immunoreactivity only for vimentin and was negative for NSE. Atypical poorly differentiated Ewing's sarcoma was immunoreactive for vimentin and NSE or a single neural marker. In contrast, PPNET (differentiated Ewing's sarcoma) reacted with vimentin and with a number of neural markers and could possess rosettes and pseudorosettes morphologically. The expression of neural markers would be expected to increase with neural differentiation from undifferentiated Ewing's sarcoma to PPNET (differentiated Ewing's sarcoma). Although differentiating rhabdomyosarcoma is possible on the basis of desmin, myoglobin, muscle-specific antigen, smooth-muscle actin, and other myogenic antibodies, always a certain small percentage of cases may have aberrant immunoreactivity. Some cases of rhabdomyosarcoma have been reported to be immunoreactive for NSE. Other neural markers, such as protein gene product 9.5, HNK-1, neurofilament triple proteins, synaptophysin, chromogranin, and S-100 protein, may provide evidence for neural differentiation in Ewing's sarcoma; however, these agents alone will not provide a definitive diagnosis.

| Marker | Ewing's sarcoma | | PPNET | Neuroblastoma | Rhabdomyosarcoma | Lymphoma[a] |
|---|---|---|---|---|---|---|
| | Typical | Atypical | | | | |
| NSE | - | +/- | + | + | +/- | - |
| S-100 | - | +/- | + | + | +/- | - |
| MFP | - | - | +/- | + | +/- | - |
| Desmin | - | - | +/- | - | + | - |
| Actin | - | - | +/- | - | + | - |
| Vimentin | + | + | +/- | - | + | + |
| Cytokeratin | +/- | +/- | +/- | - | +/- | - |
| CD | - | - | +/- | - | - | + |
| HNK-1 | +/- | +/- | + | + | + | - |
| L-Microglobulin | + | + | +/- | -/+ | +/- | + |
| HLA-71 | + | + | +/- | - | +/- | - |

PPNET, peripheral primitive neuroectodermal tumor; -, negative; +/-, positive in greater than 10% of cases; +/-, positive in less than 10% of cases; +, positive.
[a]Nonhistiocytic.
[b]Only in ganglion cells, Schwann cells, and atypical MFS.
[c]Only well-differentiated rhabdomyoblasts.

**TABLE 33-3. IMMUNOCYTOCHEMISTRY OF EWING'S SARCOMA FAMILY OF TUMORS AND OTHER SMALL BLUE-CELL TUMORS OF CHILDHOOD**

In the majority of cases, a hematopoietic malignancy may be eliminated by including leukocyte common antigen in the immunocytochemical panel. Vimentin is helpful in two ways. First, it allows determination of whether antigens have survived formalin fixation and tissue processing. Second, even though vimentin is present in many

tumors, it is absent in the vast majority of neuroblastomas and expressed fairly highly by Ewing's sarcoma.

The ESFT may be differentiated from neuroblastoma on the basis of immunoreactivity for a major histocompatibility class I antigen, b $_2$-microglobulin.[59,120,129,146] This antigen is expressed in the ESFT but is not present in neuroblastoma. Neuroblastoma may be discriminated also from Ewing's sarcoma by using monoclonal antibody NCL-NB84, which is expressed in more than 90% of neuroblastomas but has not been identified within the ESFT. [59,145]

Introduction of monoclonal antibodies that recognize glycoprotein p30/32 $^{MIC2}$ (CD99; Fig. 33-3G) has proved to be useful in confirming the diagnosis of the ESFT.[54,55,59,120,148,149,150,151,152,153,154,155,156,157,158 and 159] This cell-surface glycoprotein encoded by the pseudoautosomal *MIC2* gene on chromosomes Xp and Y is expressed to a high degree by neoplasms in the Ewing's sarcoma family. CD99 is considered to be a rather primitive marker and is highly expressed in early CD34-negative hematopoietic precursor cells. This transmembrane glycoprotein is involved with cell-to-cell adhesion during hematopoietic cell differentiation, apoptosis of immature thymocytes, and transmembrane protein transport. Roles in cell cycle progression control, cell morphology determination and differentiation, Rac-Rho signaling pathway control, and cytokinesis have been demonstrated. A number of monoclonal antibodies (HBA71, 013, 12E7, RFB1) to this glycoprotein are available and are immunoreactive with 95% to 100% of Ewing's sarcomas and PPNETs. However, this surface glycoprotein (CD99) is expressed also by other tumors and normal tissue (Table 33-4), including the nonblastomatous portions of Wilms' tumors, lymphoblastic lymphoma and leukemia, clear-cell sarcoma of the kidney, pancreatic islet cell tumors, immature teratomas, testicular embryonal carcinoma, malignant triton tumor, ependymomas, and choroid plexus papillomas. [59,153,154,156] The level and degree of expression in these tumors are considerably less than those in Ewing's sarcoma. Also, CD99 reacts with the cytoplasmic membrane of Ewing's sarcoma cells, imparting a honeycomb staining pattern (Fig. 33-3G). This contrasts with the diffuse CD99 staining of the cytoplasm in most tumors and normal tissues. Furthermore, other tumors may be differentiated readily from Ewing's sarcoma and PPNETs on the basis of morphologic, immunocytochemical, and ultrastructural features. The overexpression of this p30/32 $^{MIC2}$ protein is not related to the tumor-defining translocations in the ESFT. [54,55,59,150,151,152,153,154 and 155] However, an association between the HBA71 epitope and the growth-promoting effects of IGF-1 have been established. [158] Expression of the HBA71 antigen epitope is modulated positively by IGF-1 and insulin, resulting in cell proliferation, and modulated negatively by human growth factor and dexamethasone, resulting in growth inhibition. In contrast, HBA71 monoclonal antibody in cell cultures inhibits growth of Ewing's sarcoma and PPNET cell lines while down-regulating the IGF-1 receptor. Perhaps the overexpression of glycoprotein p30/32 $^{MIC2}$ by the ESFT may be due to the influence of growth factors, such as IGF-1 and insulin, promoting proliferation via the HBA71 epitope receptor.

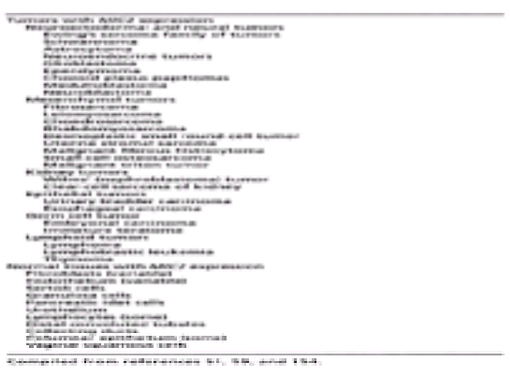


**TABLE 33-4. *MIC2* (CD99, P30/32$^{MIC2}$) EXPRESSION IN TUMORS AND NORMAL TISSUE**

A novel immunocytochemical marker for the ESFT recently was evaluated.[147] This antibody is directed toward b$_1$-integrin–linked protein kinase (ILK). With Ewing's sarcoma, an intense cytoplasmic staining pattern is present in all Ewing's sarcomas and PPNETs examined, similar to that seen with CD99 (p30/32 $^{MIC2}$). However, approximately one-third of neuroblastomas and all medulloblastomas also reacted with ILK. ILK is believed to be a possible marker of primitive neural differentiation. On the mRNA level, ILK is expressed in a wide variety of normal human tissues. This antibody does react with formalin-fixed, paraffin-embedded normal skeletal and cardiac muscle. ILK immunoreacts with well-differentiated rhabdomyoblasts but not with undifferentiated rhabdomyoblasts, which more typically are present with childhood small round-cell tumors. All lymphoblastic lymphomas and leukemias failed to immunoreact with ILK. Other tumors that may be confused with the ESFT and were negative for ILK included mesenchymal chondrosarcoma, osteosarcoma, retinoblastoma, and osteoblastoma. ILK may be a marker also for anchorage-independent cell growth and increased motility in Ewing's sarcoma. Overexpression of ILK inhibits adhesion to integrin substrates and is associated with dysregulation of integrin-mediated signal transduction. The *ILK* gene locus (11p15) is in close proximity to the *FLI1* gene locus (11q24).

The diagnosis of undifferentiated tumors in childhood very obviously requires a multimodal approach to reach a definitive diagnosis. Histopathologic, ultrastructural, and immunocytochemical information provides supporting evidence for a specific tumor entity. In addition, cytogenetic and molecular cytogenetics, including RT-PCR and FISH, supply additional information that will characterize the neoplasm further and, in some cases, provide the tumor-defining genetic abnormality. So that all avenues are available for diagnosis, it is important that tissue be set aside and properly handled for each of the diagnostic instruments.

### Histopathologic Evaluation and Prognosis

With certain tumors, pathologic variables have been associated with clinical course and survival. In the case of the ESFT, a paucity of studies that have been performed indicates a link between tissue studies and clinical outcome. [45,47,48,49,50,51,52 and 53,59,119,120,132,160,161,162,163,164 and 165] As mentioned, proliferative indices may play a role in assessing outcome; however, relatively few studies have been performed on a limited number of cases. [114,115,116,117,118,119,120 and 121] Whether DNA ploidy status or proliferative indices, such as PCNA (Fig. 33-3E, F) or Ki-67 expression, will prove to be beneficial will depend on well-controlled longitudinal studies.

In the past, classification of the ESFT [45,120] into typical undifferentiated Ewing's sarcoma, atypical poorly differentiated Ewing's sarcoma, and PPNET (differentiated Ewing's sarcoma) has been shown to be an independent prognostic factor in patients with localized primary tumors of distal extremities. Patients with tumors defined to be typical Ewing's sarcoma had a significantly improved overall survival as compared with those with either atypical Ewing's sarcoma or PPNET. Survival with localized PPNET was decreased significantly as compared with either typical or atypical Ewing's sarcoma. Although these results apply only to localized disease, histopathologic evidence of metastatic disease was identified as a significant independent factor in decreased survival.

This particular clinicopathologic study reported metastatic disease in approximately 10% of typical Ewing's sarcomas, whereas metastases were identified in slightly more than 35% of atypical Ewing's sarcoma and PPNET. Even though these results are derived from a limited number of cases with primary tumors of distal extremities, which had undergone different oncologic and surgical management, with the ESFT, a progression has been implied to more aggressive biologic behavior with increasing neural differentiation. Similar findings regarding neural differentiation and links to prognosis and clinical outcome also have been reported. [47,48,49,50,51,52 and 53,132] In fact, attempts to link the immunoreactivity of certain polyclonal and monoclonal markers to biologic behavior have shown that expression of a certain neural marker (HNK-1, Leu-7) may be associated with more aggressive behavior, whereas another neural marker (S-100 protein) may be linked to a more favorable outcome.[47] The findings in these studies are suspect, owing to the inclusion of many cases that had not undergone similar surgical or oncologic management.

More recently, comprehensive evaluations of the ESFT receiving uniform oncologic management found that neuroectodermal differentiation did not predict tumor behavior. These evaluated clinicopathologic studies determined neural differentiation by immunocytochemical (CD57, S-100 protein, neurofilament protein, and synaptophysin) and ultrastructural means.[148,149] Electron-microscopical evaluation significantly increased the likelihood of classifying tumors as PPNETs and was considered to be the optimal method for defining neuroectodermal differentiation. However, no significant difference in 5-year EFS or overall survival in patients with localized or metastatic disease was identified between those with and without neural features. At present, the overall consensus is that neuroectodermal differentiation does not affect outcome and should not direct therapy in the ESFT.

### Histologic Response to Therapy and Prognosis

Of particular interest is determination of the prognostic significance of histopathologic response to preoperative chemotherapy in individuals with resectable Ewing's sarcoma tumors.[120,160,161,162,163,164 and 165] The relative percentage or grade of tumor necrosis and fibrosis after preoperative chemotherapy appears to be related to

clinical outcome. Three separate histologic response grading systems have been used. [120,160,161 and 162] The first uses a three-tiered grading (Picci) system. [160,161] GradeI histologic response is assessed when macroscopic foci of viable tumor cells composed of large individual nodules or smaller scattered nodules occupy an area greater than a 10× objective magnification field. Grade II histologic response is defined as isolated microscopic foci of viable tumor cells that occupy an area less than a 10× objective magnification field. Grade III histologic response occurs when no viable tumor can be identified.

At surgical resection, the distribution of histologic response to induction chemotherapy has been found to be 37.5% grade I (poor responder), 24% grade II (good responder), and 38.5% grade III (good responder). EFS after 5 years for localized disease was 82% for grade III, 74% for grade II, and only 28% for grade I ( $p$ <.001 between grades I vs. grades II and III). With those who died of relapsed disease, survival time was 51 months for good responders (grades II and III) as compared with 33 months for poor responders (grade I; $p$ <.03). By both univariate and multivariate analyses, this histologic response grading system proved to be a prognostic factor in survival, with a 2.6-fold decrease in EFS for individuals with poor histologic response ( $p$ <.001).

The second histologic response grading method used the existing four-tiered semiquantitative (Huvos) grading system for osteosarcoma treatment effect. [162] Grade I is tumor necrosis of less than 50% of the tumor. Grade II is tumor necrosis of more than 50% to less than 90%. Grade III is tumor necrosis of 90% to 99%. Grade IV is tumor necrosis of 100%. After induction chemotherapy and surgical excision, the distribution of histologic response was 19% grade 1, 22% grade II, 18% grade III, and 42% grade IV. EFS at 5 years was 0% for grade I histologic response, 37.5% for grade II histologic response, and 84% for grades III and IV histologic response ($p$ = .0001). No significant difference in survival could be determined between grade III and grade IV responders (good histologic responses). EFS was significantly different between grades III and IV as compared with grades I and II ($p$ = .0001) and also between grades I and II ($p$ = .007). The relative risk for systemic recurrence was increased by 25.0-fold and 8.3-fold for grade I and grade II, respectively, with tumors smaller than 8 cm in maximum dimension, when compared with grades III and IV.

The third grading method was used in the Pediatric Oncology Group–Children's Cancer Group (POG-CCG) intergroup Ewing's sarcoma study and uses a modified Huvos system (grade IV, 100% tumor necrosis; grade III, 91% to 99% necrosis; grade IIB, 11% to 90% necrosis; grade IIA, 1% to 10% necrosis; grade I, no necrosis).[120] Survival at 3 years was 100% for grade IV, 73% for grade III, 49% for grade IIB, and 30% for both grades IIA and I.

The importance of histologic response assessment to chemotherapy after surgical resection is illustrated by these studies. Evaluation of chemotherapy-induced necrosis is of prognostic value and may help to direct further oncologic therapy. Current studies are attempting to compare chemotherapy response by dynamic magnetic resonance imaging (MRI) with histologic response after resection. If dynamic MRI correlates well with histologic response, it may provide a method by which to assess chemoresponsiveness, to identify poor responders during therapy, and to alter the oncologic regimen to effect an improved response.

## CLINICAL FEATURES

### Primary Sites

Data compiled from 18 studies published over a 10-year period in the United States, Europe, and Japan included 1,505 patients with the ESFT.[27,166,167,168,169,170,171,172,173,174,175,176,177,178,179,180,181 and 182] The data compiled from these patients with the ESFT revealed that the primary sites were divided almost evenly between the extremities (53%) and the central axis (47%). The extremity tumors were distal in 52% and proximal in 48%. The central axis tumors were of the pelvis (45%), chest wall (34%), spine or paravertebral region (12%), and head or neck (9%). The 74 PPNETs most commonly were of the central axis (74%); of these, most arose in or around the chest (60%). EES occurred in the extremities in 36% of cases and in central locations in the remainder. Often, determining whether a tumor is of bony or soft tissue origin is difficult, because the bony ESFT characteristically have extensive soft tissue components, and soft tissue tumors may invade bone secondarily.

### Signs and Symptoms

At diagnosis, signs and symptoms are both constitutional and related to the sites of disease. Pain or swelling (or both) at the site of the primary tumor most often are the presenting symptoms. Of 140 patients treated at the Mayo Clinic for Ewing's sarcoma of bone, 96% presented with pain, 61% with a palpable mass, 16% with a pathologic fracture, and 21% with fever.[166] Of 42 EES patients reported by the Mayo Clinic, 75% presented with a palpable mass and 66% with pain. Additionally, hemorrhage and necrosis are seen commonly within the ESFT, which may result in localized warmth and edema. Discerning Ewing's tumor from infection can be difficult because of these symptoms.

Patients can present with symptoms from their metastatic sites rather than pain at the primary site. Back pain may represent the first symptom of spinal cord compression secondary to a primary or metastatic spine tumor and requires an emergency evaluation and therapy before irreversible neurologic damage develops. Primary or metastatic tumors to the bony pelvis may cause leg pain secondary to peripheral nerve involvement. Bony metastasis may be palpable on the skull, ribs, or any superficial bone. Unexplained fevers may be the first sign of disease recurrence.

Often, a delay in Ewing's sarcoma occurs between the first symptoms and diagnosis. Forty-eight percent of 331 patients treated on the Intergroup Ewing's Sarcoma Study 1 (IESS-1) study had symptoms for more than 3 months before diagnosis.[183] In a report from Denmark, the duration of symptoms before diagnosis averaged 9.6 months, with a range of 4.0 weeks to 4.0 years.[184] One reason for the delay may have been that the pain was intermittent in 68%, providing false reassurance to physicians, patients, and family. Delay was much shorter in those with a palpable mass than in other patients (3.2 versus 10.1 months, respectively). Often, a delay can occur in the diagnosis of pelvic tumors, because they frequently are not palpable until the tumors are fairly large.

### Patterns of Spread

The high rate of distant failure (more than 80%) noted in patients who received local treatment alone before the advent of systemic chemotherapy supports the notion that most patients with the ESFT have, at a minimum, microscopic metastases at diagnosis. Approximately 25% of patients with the ESFT present with overt metastases at diagnosis. Metastases primarily follow the hematogenous route, although metastases through direct extension can occur. The most common sites of metastases are the lung (38%), bone (31%), and bone marrow (11%).[185] The spine is one of the more common sites of bone metastasis.[166] Lymph node (7%) and liver metastases are uncommon.[178] Intra-abdominal metastases to the peritoneum or gastrointestinal tract have been reported in EES. [173] Metastases to the central nervous system are rare, occurring in fewer than 5% of cases. A recent review at St. Jude Children's Research Hospital of all patients treated for Ewing's sarcoma over a 36-year period revealed that of 335 Ewing's sarcoma patients, 11 (3.3%) had brain metastases. [186] All these patients demonstrated clinical neurologic signs. Historically, prophylaxis with radiotherapy and intrathecal methotrexate does not prevent central nervous system metastases from occurring. [187] Patterns of metastasis are more likely a function of the primary site than of the specific histology. For example, a common site of spread for primary ESFT of the chest wall is to the adjacent pleural space.

### Differential Diagnosis

If Ewing's sarcoma or PPNET presents as a tumor of bone, the differential diagnosis includes a variety of benign and malignant processes. An important consideration is osteomyelitis, especially if an affected patient is febrile. Benign tumors of bone that can present as a lytic bone lesion include eosinophilic granuloma and giant-cell tumor. Malignant tumors that should be considered include osteosarcoma, primary lymphoma of bone, spindle cell sarcomas of bone (e.g., malignant fibrous histiocytoma), and metastases from a nonbone tumor, especially neuroblastoma. Neuroblastoma can present with an asymptomatic abdominal or thoracic mass, which may go undetected, and a single symptomatic bony metastasis. EES and PPNET must be differentiated from benign and malignant soft tissue tumors (see Chapter 32 and Chapter 34).

### Evaluation

The evaluation of a patient with a lesion that is suspected or confirmed to be in the ESFT has four goals. First, the full extent of the primary tumor must be defined as accurately as possible through the use of a variety of imaging techniques and histologic sampling. Second, a metastatic workup should determine whether clinically evident metastases are present and should define the sites and extent carefully. Third, because the patient is likely to undergo rigorous treatment, a physiologic evaluation should determine baseline cardiovascular, pulmonary, renal, and hepatic functioning. Fourth, any neurologic, musculoskeletal, or psychological problems that may interfere with the patient's rehabilitation must be identified so that appropriate interventions are initiated as early as possible.

### *Laboratory Studies*

Neither a blood test nor a urine test provides specific markers of the ESFT. Laboratory studies should include a complete blood count, baseline chemistries, and an erythrocyte sedimentation rate analysis. An elevated erythrocyte sedimentation rate can be found in up to 50% of cases. [188] Because an elevated serum lactate dehydrogenase level has been demonstrated to have adverse prognostic significance, a baseline analysis should be obtained. [189] If neuroblastoma is in the differential diagnosis, urine catecholamine levels may be useful because they are normal in the ESFT but usually elevated in neuroblastoma.

Bilateral iliac bone marrow aspirations and biopsies should be performed to evaluate the bone marrow for metastatic disease. MRI is sensitive to abnormalities within the marrow, but the changes seen are not specific. Any decrease in fat within the marrow may alter the signal, creating difficulty in differentiating tumor from increased hematopoiesis.

Gene fusions that result from the nonrandom chromosome translocations found in the ESFT provide tumor-specific markers that can be used to detect the presence of the tumor cells. RT-PCR allows detection of tumor cells in peripheral blood, bone marrow, and stem-cell collection. This approach permits the detection of one tumor cell in more than $10^6$ mononuclear cells, as shown by serial dilution of Ewing's cells in control peripheral blood. [190] RT-PCR provides an extremely sensitive and specific method for molecular staging and monitoring treatment response. Zoubek at al. [101] confirmed an association of bone marrow positivity by RT-PCR with bone or bone marrow metastasis. Additionally, one-third of this study's patients with localized disease had bone marrow positivity on RT-PCR testing. Preliminary statistical evaluation suggested a nonsignificant tendency toward adverse outcome in this group of patients with localized disease. [101] Several researchers have demonstrated that minimal disease can be detected in bone marrow and peripheral blood, although at present how these factors may contribute to patient management remains controversial.[99,191] Another important application of RT-PCR is in the testing of stem-cell collections before transplantation. By using RT-PCR, peripheral blood stem-cell harvests have been found to be contaminated with tumor cells. [192]

### *Diagnostic Imaging*

The diagnostic imaging evaluation should consist of a thorough examination of the primary site and a search for metastatic disease ( Fig. 33-5, Fig. 33-6, Fig. 33-7, Fig. 33-8, and Fig. 33-9). The importance of fully defining the extent of disease at the primary site before therapy cannot be overemphasized. The planning and delivery of optimal local therapy require documentation of the initial extent of the tumor, which can be expected to decrease after chemotherapy. Plain films, MRI of the primary site, computed tomography (CT) of the chest, and bone scans are necessary for the staging work-up for the ESFT.

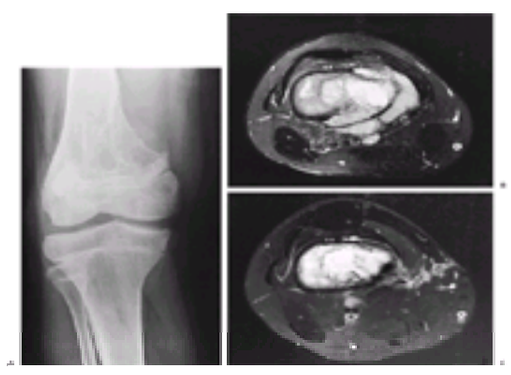


**FIGURE 33-5. A:** Plain radiograph of distal femur showing involvement of the metaphysis by Ewing's sarcoma, with significant destruction of cortical bone and soft tissue swelling. **B:** Magnetic resonance image through distal femur of same patient at time of presentation, again demonstrating femoral involvement by tumor and adjacent soft tissue mass. **C:** Magnetic resonance image through distal femur of same patient after 12 weeks of chemotherapy with vincristine, Adriamycin, and cyclophosphamide alternating with ifosfamide and etoposide. Note regression of soft tissue mass. (Courtesy of T. Chung, M.D., Baylor College of Medicine, Houston, TX.)

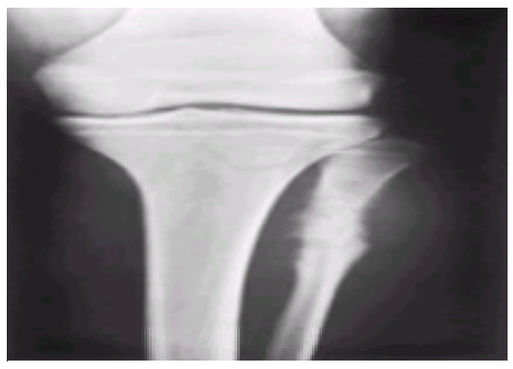


**FIGURE 33-6.** Ewing's sarcoma of the fibula, a favorable site for resection. A wide excision with negative margins can often be obtained.

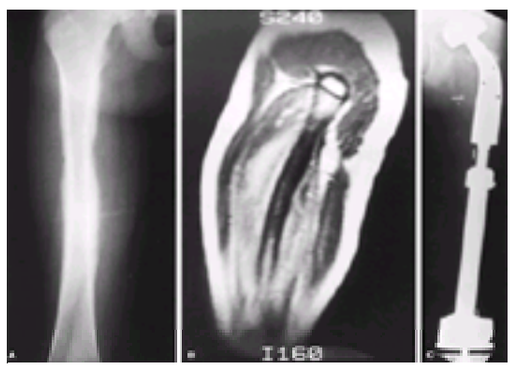



**FIGURE 33-7. A:** Plain radiograph of Ewing's sarcoma of the femur occurring in a 10-year-old child. **B:** Magnetic resonance image of the femur demonstrating almost total intraosseous involvement and a large extraosseous component. **C:** Postoperative radiograph after resection of the entire femur and replacement with an expandable total femoral prosthesis.

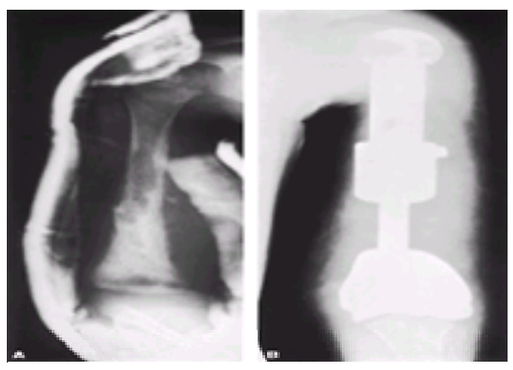


**FIGURE 33-8. A:** Plain radiograph of Ewing's sarcoma of the humerus in a 4-year-old patient. This was treated by induction chemotherapy followed by resection of the entire humerus. All margins were negative. Radiation was not used because of the child's age. **B:** Postoperative radiograph showing an expandable total humeral prosthesis. (Courtesy of J. Eckardt, M.D., University of California, Los Angeles, CA.)

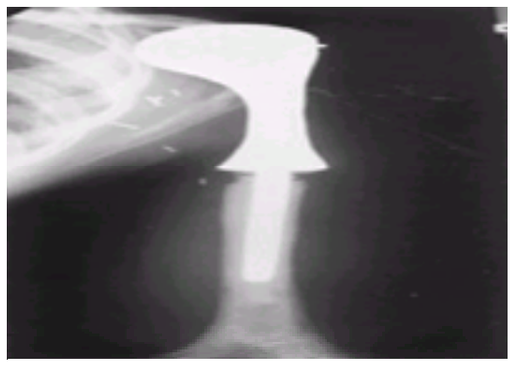


**FIGURE 33-9.** Plain radiograph after resection and replacement of the proximal humerus for a large Ewing's sarcoma. This patient was treated with a combination of preoperative chemotherapy and radiotherapy. The radiograph shows the result at 8 years after treatment.

The radiograph of a Ewing's tumor of bone usually reveals a poorly marginated, destructive lesion of the diaphysis of bone. [193,194] The erosion of the cortex and spread to the soft tissues surrounding the bone sometimes are accompanied by a multilaminated periosteal reaction (i.e., onion peel); the Codman's triangle of elevated periosteum may or may not be seen. Surrounding soft tissue mass often is disproportionately large as compared to the bony component. A sclerotic appearance may be present, especially in flat bones. Sclerosis is secondary to bony reaction, not tumor bone formation as is seen in osteosarcoma. However, distinguishing the ESFT and osteosarcoma radiologically is difficult. A plain radiograph with a marker or ruler to correct for magnification of the entire bone should be obtained to make the accurate measurements needed for limb-sparing resections and prosthesis if they are being considered.

MRI is the method of choice in the local staging of Ewing's sarcoma at diagnosis. [193,195] MRI displays the tumor in multiple planes and, with soft tissue contrast, can assess the intramedullary and extramedullary tumor extent. This is critical with regard to planning of (limb-salvage) surgery and irradiation. MRI is important in determining the relationship of the tumor to the adjacent physis, muscle compartments, joint, and neurovascular structures. [193] T1-weighted images are particularly accurate in assessing the intramedullary tumor extent. On T1-weighted images, the low signal intensity of tumor contrasts with the high signal intensity of the fatty bone marrow. Moreover, sagittal-coronal T1-weighted spin echo MRI has a sensitivity higher than that of transaxial CT, conventional radiography, and bone scintigraphy for the detection of intramedullary skip lesions in patients with Ewing's sarcoma. [196,197] T2 weighting displays both the interface between adjacent soft tissues and tumor and the anatomic relationship with the neurovascular structures. This allows differentiation between intracompartmental and extracompartmental disease.[197] MRI is superior in discerning between tumor and vascular structures. [193] Thus, angiography is not advocated any longer as a routine staging procedure for Ewing's sarcoma. In terms of identifying cortical bone involvement, this usually can be seen on a plain film, and large cortical lesions can be seen on MRI. If it is clinically necessary to determine whether a patient has a small cortical break, CT of the primary tumor may be necessary.

Besides local staging at diagnosis, MRI has an important role in monitoring the effects of chemotherapy. Most Ewing's sarcomas are accompanied by a substantial soft tissue mass seen on MRI. After chemotherapy, a significant decrease in tumor volume will be found in the majority of patients with Ewing's sarcoma. Additionally, changes in signal intensity within Ewing's tumors have been evaluated to allow prediction of response to chemotherapy. Fast dynamic contrast-enhanced MRI studies can distinguish good from poor responders.[193] With this technique, after a bolus injection of gadolinium contrast, an increase in signal intensity is plotted as a function of time. A time-intensity curve is produced, and a steeper slope presumably corresponds to more rapid uptake of the contrast agent, owing to increased vascularization and perfusion associated with viable tumor. [193] In addition, dynamic studies may help in the differentiation between residual tumor and reactive peritumoral edema. Compared with edema, viable tumor is characterized by an earlier, higher, and more rapid uptake of contrast agent. [193]

Distant metastases from Ewing's sarcoma most often become manifest in the lungs. CT is superior to conventional radiography for the detection of lung metastases. A routine CT scan of the chest to screen for pulmonary metastases at time of diagnosis is essential for early detection and is of critical value for prognosis.

Radionuclide bone scanning with technetium 99m methylene diphosphonate is used to define the bony extent of the tumor and to screen for metastases to distant bone. A bone scan must be performed at diagnosis to stage all Ewing's sarcoma patients accurately.

### *Initial Radiotherapeutic Evaluation*

Radiation oncologists should evaluate affected patients at the time of initial diagnosis, perform a physical examination, and review the laboratory and radiographic studies before therapy. Special attention should be given to the clinical features of such patients and to the tumor to determine suitability for delivery of radiotherapy. These factors include the patient's age, tumor site, tumor size, soft tissue extent of the tumor, extent of medullary cavity involvement in the affected bone, relation of the tumor to the epiphyseal plates and joints (in growing children), the possibility of sparing adjacent soft tissues and viscera from the high-dose region of the radiation field, the presence of pathologic fracture, and the functional status of the involved joint (especially limb) at the time of presentation. Radiation oncologists certainly should discuss with their surgical colleagues the feasibility of a complete resection and the probable functional outcome of the surgery before recommending radiotherapy as the principal local treatment.

### *Initial Surgical Evaluation*

Surgical resection, if used, usually follows neoadjuvant chemotherapy or radiotherapy (or both). [179,198,199,200,201 and 202] Nevertheless, surgeons who may be asked to intervene later should participate in the initial evaluation of affected patients to estimate and stage the local tumor extent and to perform the initial biopsy. Whenever possible, biopsies of suspected bone malignancies should be performed at the center at which the ultimate treatment will be rendered, as an improperly executed biopsy may render limb preservation difficult if not impossible. A poorly planned biopsy of a bony lesion may result in pathologic fracture or may contaminate other tissue planes, rendering resection difficult. Multiple cores or, preferably, an open biopsy should be performed to obtain enough material for diagnosis. Fine-needle aspiration cytology is not acceptable as the only diagnostic material and should be used only to sample metastatic sites or suspected sites of recurrence when the histologic diagnosis is known. Frozen-section analysis of all biopsy specimens is essential to determine whether adequate tumor has been obtained to yield satisfactory diagnostic material. Material is necessary for electron-microscopical studies, immunohistochemistry, RT-PCR, and cytogenetics. It is not unusual for a large portion of the tumor to be necrotic and thus nondiagnostic.

The biopsy site should be chosen carefully and placed in line with any potential resection incision site and radiation portals. If the ESFT is suspected, the biopsy

specimen should, if possible, be taken from the extraosseous component rather than from the underlying bone. A bony defect may not reossify if irradiation is required and is more likely to fracture after radiotherapy. If biopsy of the underlying bone is necessary, a small, rounded cortical window should be made. Regardless of the technique used, tumor cells contaminate all tissue planes and compartments traversed, and all biopsy sites, therefore, must be included en bloc when the tumor is irradiated or resected.

Because irradiation is included in the treatment of many patients with the EFST, it is important to remember that irradiated scars can be vulnerable to trauma. Scars placed directly over bone (e.g., tibial shin) are more prone to radiation injury than those placed in more richly vascularized soft tissue or connective beds. [203] Smaller biopsy incisions are less likely than larger incisions to break down after radiotherapy and chemotherapy.

Ewing's sarcoma can mimic osteomyelitis clinically and radiographically; for this reason, surgeons responsible for treating osteomyelitis should perform the incision and drainage with the idea that an affected patient might have a malignant bone tumor. Routine bacterial cultures should be obtained in case the lesion proves to be an osteomyelitis.[204]

### *Staging*

Although no commonly used staging systems exist for the ESFT, treatment protocols often are stratified for the presence or absence of metastatic disease and according to primary site. A useful pretreatment staging system would be based on the presence or absence of metastatic disease and on size, extent, and location of the primary lesion, similar to the system recently developed for rhabdomyosarcoma (see Chapter 32). Because a staging system would be helpful in comparing the results of clinical trials, it is hoped that such a system can be developed that will be accepted internationally.

## TREATMENT

### General Principles

The treatment goal for the ESFT is to cure the disease while preserving the patient's function and minimizing long-term sequelae. Approaching with noncurative intent a child with newly diagnosed ESFT disease is rarely, if ever, appropriate, because one cannot determine a priori whose disease will be cured. Even if cure is not realized, treatment usually results in tumor regression, which may translate into prolonged survival and an improved quality of life. Optimal outcome requires the close cooperation of members of a therapeutic team experienced in the treatment of these disorders. For this reason, the ESFT is best managed, from biopsy onward, in pediatric medical centers. Whenever possible, patients should be entered into a clinical research protocol.

ESFT is a systemic disease. Although most patients have no clinical evidence of metastatic disease at diagnosis, it must be assumed that every patient has microscopical metastases. Treatment is often conceptually compartmentalized into measures to effect either local or systemic control, but this is an oversimplification. Most modern treatment plans are neoadjuvant in design. Systemic chemotherapy, in addition to being the mainstay of treatment for microscopical and gross metastatic disease, usually is effective in reducing local tumor volume. Reduction of tumor mass may increase the likelihood of local control with radiotherapy and facilitate a surgical resection. Local control measures should not be allowed to compromise systemic therapy, as treatment failures usually are attributable to the development of distant metastatic disease. Surgical procedures that result in a protracted postoperative course and interruption in chemotherapy are likely to have an adverse effect on systemic control. For purposes of this discussion, we too compartmentalize therapy into local and systemic forms, but we emphasize strongly the critical nature of the interactions between these modalities.

### Local Control: General Considerations

In 1921, James Ewing[1] observed that “small-cell sarcoma of bone,” unlike osteosarcoma, was responsive to a radium implant. In the ensuing four or five decades, we came to recognize that although surgery and radiotherapy could control the local disease, more than 90% of patients eventually died from metastatic disease. [205,206] Local control of the primary tumor with radiotherapy ranged from 50% to 77%.

With the introduction of effective multi-agent chemotherapy, not only did the overall survival rates of patients increase because of eradication of microscopic metastatic disease, but an improvement in local control of the primary tumor was observed after radiotherapy. [176,184,207,208 and 209] In the IESS-1, the use of doxorubicin was shown to increase the local control rate. [209] Local control rate after chemotherapy and radiotherapy usually ranges from 75% to 90% ( Table 33-5).[207,208 and 209]

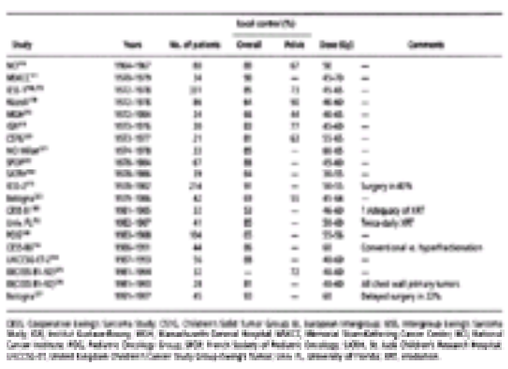


**TABLE 33-5. PRIMARY TUMOR CONTROL IN PATIENTS WITH LOCALIZED EWING'S SARCOMA TREATED BY IRRADIATION TO THE PRIMARY SITE IN CONJUNCTION WITH MULTI-AGENT CHEMOTHERAPY**

Factors other than chemotherapy probably also were responsible for the apparent improvement of local control after radiotherapy in the last two decades. These factors include more common use of CT and MRI to define the extent of tumor better and improved radiotherapy delivery techniques. The importance of technique is illustrated by the Cooperative Ewing's Sarcoma Study (CESS-81) of the German Cooperative Group. In the initial phase of this trial, the relapse-free survival rate of patients treated with radiotherapy was 50%, which was significantly lower than that for surgical therapy. [210] On review, the high rate of local failure after radiotherapy was largely attributed to geographic miss. After the initiation of a quality assurance program in 1984, the local control rate improved significantly, and the relapse-free survival rate increased to 80%.

Local control analysis in Ewing's sarcoma can be complicated by the difficulties encountered both in interpreting the diagnostic studies after combined-modality treatment and in assessing local control in patients who experience relapse with widely metastatic disease. Bone involved with tumor that is subsequently treated with chemotherapy and irradiation undergoes healing and remodeling. Regression of soft tissue masses and resolution of clinically evident swelling, pain, tenderness, and erythema accompany this healing. On plain radiographs, a predictable series of changes occur over approximately a year, eventually stabilizing and remaining constant in appearance unless relapse or second malignancy occurs. [174] However, the plain films, bone scans, CT scans, and MRI scans rarely return to normal after treatment. Changes in MRI signals of tumor-involved sites after chemotherapy or radiotherapy still are difficult to interpret definitively. Large tumors with significant soft tissue components often regress incompletely, with residual soft tissue masses on imaging studies. These abnormalities on imaging studies may represent tumor, fibrosis, necrosis, or osteomyelitis. Time-intensity curves derived from gadolinium-enhanced MRI studies correlate better with histologic response than do changes in tumor size.[193] Because of the potential morbidity from biopsies of irradiated bone and soft tissues, stable abnormalities on imaging studies should be monitored closely. Radiographic or clinical evidence of progression warrants biopsy of the area of abnormality. In patients who experience relapse with metastatic disease, the primary site is not necessarily reevaluated to assess local control unless it becomes symptomatic. Detection of local recurrence in such patients depends in part on the thoroughness with which the primary site is reevaluated by imaging or biopsy. An autopsy series from the National Cancer Institute (NCI) demonstrated an unexpectedly high proportion of patients (13 of 20) with tumor in the irradiated site after initial treatment with irradiation and chemotherapy. [202] The clinical relevance of this observation is not completely clear. The tumor cells in the irradiated area may have represented a local recurrence or reseeding of the primary site from widely metastatic tumor, or nonclonogenic cells. Despite this observation, the local control figures from the NCI series show only a 17% frequency of clinically evident local

recurrence.[211]

### Surgery versus Radiotherapy

Both surgery and radiotherapy are effective local treatment for Ewing's sarcoma. The relative roles of surgery and radiotherapy continue to be controversial. Because no randomized study has compared the two modalities directly, only a relative comparison can be made from retrospective studies, keeping in mind the various admixtures of known prognostic factors in the different series. Several reviews, including those from the Memorial Sloan-Kettering Cancer Center, [212] Mayo Clinic,[166] and Massachusetts General Hospital,[213] have suggested that surgery plus chemotherapy produces either better rates of local control or better survival rates than does radiotherapy plus chemotherapy. However, all these reports included smaller lesions in more favorable locations in the surgical group as compared to larger central and inoperable lesions in the radiotherapy group, thereby biasing the results in favor of surgery. In addition, some series (e.g., the series from Memorial) incorporated postoperative radiotherapy in some of the surgically treated patients. Similarly, 116 Ewing's sarcoma patients from a single institution were retrospectively reviewed by Aparicio et al.[214] and, although the patients undergoing surgical resection had a superior DFS, these patients often had favorable prognostic features (i.e. normal LDH, small tumors, local disease, and nonaxial site).

Recently, the M. D. Anderson Cancer Center reported their 20 years of experience with nonmetastatic Ewing's sarcoma, which included 85 patients. [215] All but 1 of the 85 patients received treatment with combination chemotherapy. All patients received x-irradiation as local treatment, either solely or in addition to surgical resection. A total of 20 patients underwent surgical resection as part of their local treatment. Patients who received surgery as part of the planned treatment of their primary tumor had significantly better local control and DFS than those who did not undergo resection. The authors point out that although this was not evaluated in a randomized study, it appeared that the patients who were subjected to surgery generally had large tumors.

In the aforementioned German CESS-81 trial, the local failure rate in the radiotherapy group was initially high owing to geographic misses. Because of the introduction of a central quality assurance program, the local control rate improved significantly. In the subsequent study, CESS-86, the 5-year relapse-free survival rates after chemotherapy plus radiotherapy and after surgery plus chemotherapy were comparable (67% and 65%, respectively).

It is generally agreed that dispensable bones such as fibula, rib, and small lesions of hands or feet are amenable to conservative surgery, especially in younger patients in whom radiotherapy could cause major growth and functional deficits. However, when a tumor is unresectable or to avoid a mutilating surgery, irradiation is a better option for local treatment. A large lesion that fails to respond to induction chemotherapy may also be considered for surgery and postoperative radiotherapy. Any lesions that are not controlled by chemotherapy plus radiotherapy are candidates for surgical salvage. [216] In making the ultimate decision regarding a local control strategy for each patient, the physician must weigh the imperative of attaining complete tumor eradication against the goal of maximizing function. An additional consideration is the likelihood of development of a second malignancy in the irradiated site within 20 years after treatment. These second malignancies usually are osteosarcomas that occur in previously irradiated bone. The incidence of bone tumors in the irradiated field has varied from study to study, with estimates at 20 years after therapy ranging from 10% to 30%.[19,217]

### Surgical Management

#### *General Principles*

The surgical treatment of Ewing's sarcoma consists of two distinct and opposed phases: adequate resection of the residual gross disease from the affected bone and soft tissue and reconstruction of the resulting injury to the musculoskeletal system. The goals of surgical treatment are complete extirpation of the primary tumor and restoration of the maximum possible level of function possible. When surgical treatment is selected for the patient with Ewing's sarcoma, the surgeon must plan an adequate resection margin separately and before he or she considers reconstruction options. The temptation to compromise tumor margins to facilitate limb-sparing surgery must be resisted.

The surgical principles applicable to the treatment of Ewing's sarcoma are similar to those followed in the treatment of osteosarcoma; however, three salient differences characterize the approach to Ewing's sarcoma: (a) Ewing's sarcoma is radiosensitive, whereas osteosarcoma is not; (b) Ewing's sarcoma tends to occur in a younger population than osteosarcoma; and (c) Ewing's sarcoma tends to arise in the diaphysis of long bones, whereas osteosarcoma has a predilection for the metaphyseal area.

The fact that Ewing's sarcoma is highly radiosensitive affords a reasonable and, in many cases, preferable, option for control of the primary tumor. [218] Radiotherapy obviates the need for a surgical incision and implants, with the consequent risk of infection. Indeed, the classical teaching has been that surgery is reserved for Ewing's sarcoma arising in “expendable” bones (i.e., fibula, clavicle, and rib) and that radiotherapy is the treatment of choice for all other locations. However, several disadvantages of radiotherapy have broadened the indications for surgery somewhat. The major problem with irradiation is the potential for late development of high-grade malignant tumors in the treatment field in approximately 15% to 30% of patients who survive their Ewing's sarcoma. [219] Postradiation sarcoma carries an extremely poor prognosis. Late disability owing to arthrofibrosis in irradiated joints, fibrosis and contracture of radiated muscles, lymphedema, and growth disturbance has been minimized, but not completely eliminated, with modern orthovoltage equipment and ultrafractionation dosage schedules. [220] Ewing's sarcoma arising in some anatomic locations, most notably the pelvis and spine, may require a combination of surgery and radiotherapy to optimize local control.

The fact that Ewing's sarcoma tends to occur in a younger patient with greater potential for skeletal growth poses a challenge to the surgeon responsible for reconstruction. When a physeal plate must be resected, the surgeon must address the potential for significant limb-length discrepancy in the process of planning treatment. This problem is especially significant when the distal femoral or proximal tibial growth plates are involved. Although expanding prostheses are available, the essential problem with long-bone growth is not longitudinal but circumferential enlargement. [221] All the currently available prosthetic devices are secured using sterns, which are either press-fit or cemented into the intramedullary canal of the affected bone. As the radius of the bone increases, the endosteal surface grows away from the implant, resulting in loosening. For this reason, implants requiring intramedullary fixation have limited application in young children in whom significant growth remains.

The third distinguishing feature of Ewing's sarcoma—the fact that it is more likely to arise in the central portion of bones—somewhat offsets the difficulties presented by skeletal reconstruction in the growing child. In cases in which the tumor is within no more than 4 cm of the adjacent growth plate, one can successfully excise the tumor-bearing bone with preservation of the physis. The surgeon may use a diaphyseal segment of allograft bone [222] or vascularized fibular autograft [223] to reconstruct the resulting bony defect.

#### *Principles of Surgical Resection*

Ewing's sarcoma frequently presents with a large mass extending into the soft tissues surrounding the involved bone. This mass is composed of tumor cells and reactive host inflammatory cells. The portion of the mass consisting of tumor cannot be distinguished radiographically from the inflammatory response. Typically, the mass rapidly resolves during preoperative chemotherapy. The MRI scan obtained as part of the staging workup before initiation of chemotherapy is important for planning the level of bone resection. The MRI scan obtained immediately preoperatively generally is used to plan the soft tissue resection. The entire length of the involved bone must be imaged preoperatively to rule out skip metastasis.[224] Rarely, the soft tissue mass will continue to enlarge on chemotherapy. In these cases, amputation may be necessary.

In the past, musculoskeletal oncologists have considered pathologic fracture through a Ewing's sarcoma an absolute indication for amputation. The rationale for this policy was that tumor cells are carried along tissue planes by the fracture hematoma, making adequate surgical resection impossible. A recent study demonstrated that minimally displaced or nondisplaced pathologic fractures may be resected using standard limb-sparing approaches without an increased incidence of local recurrence.[225]

#### *Principles of Surgical Reconstruction*

Reconstruction is not always possible or desirable after surgical removal of Ewing's sarcoma. A recommendation for amputation over limb salvage is most often prompted by a finding of (a) tumor invading major nerves, (b) inability to achieve acceptable reconstruction in the very young patient, or (c) tumors of the distal leg or foot. The guidelines for limb salvage after resection of one or more major nerves are site-specific and are discussed later. As previously noted, existing methods of prosthetic or allograft bone replacement are associated with a high failure rate in the rapidly growing young child. For tumors of the distal tibia or foot, function after

below-knee amputation is superior to the results obtained by limb-sparing surgery. Tumors of the distal fibula may be treated with conservative resection. [226]

### *Special Considerations by Anatomic Site*

#### Spinal Column

Ewing's sarcoma of the spinal column usually originates in the vertebral body and may extend into the posterior elements. Achieving an acceptable surgical margin (minimum 3-cm bone margin) is extremely difficult, if not impossible, under these circumstances. For this reason, radiotherapy either alone or in conjunction with surgery is the preferred method of local control in the spine. [227] Surgery involves resection of the entire vertebral body. Reconstruction is achieved using bone graft with spinal plates. Vascularized bone graft is preferred in a heavily irradiated area. Regardless of local treatment employed, Ewing's sarcoma of the spine carries a significantly worse prognosis than does Ewing's sarcoma of the appendicular skeleton. [228]

#### Pelvis

From the standpoint of reconstruction, the pelvis is divided into three zones. Zone I is the ilium, zone II the acetabulum, and zone III the ischium and pubis. Resection of either zone I or III in isolation requires no reconstruction. When the acetabulum is excised, either alone or in conjunction with zone I or III, reconstructive options include fusion of the femur to the pelvic remnant, allograft pelvic reconstruction, and leaving the femur free (“flail hip”). The latter approach ends in a very poor functional result with a grossly shortened extremity but is associated with the lowest risk of perioperative complications. Pelvic allografts carry a high risk of complications—infection, nonunion, and fracture—and may not be suitable in the very young child. [229,230] Additionally, allografts should generally be avoided in a previously irradiated area, because the risk of allograft fracture and nonunion is unacceptably high in this setting. Fusion provides a stable platform for ambulation and, when it is technically feasible, is probably the best choice for the young patient with Ewing's sarcoma of the pelvis. In light of the high complication rate and predictably poor functional outcome after surgery, radiotherapy should be strongly considered for local control of type II pelvic Ewing's sarcoma. Surgery, with or without radiotherapy, has been shown to be associated with no higher risk of local recurrence than radiotherapy alone. [231] Ewing's sarcoma of the pelvis is associated with a poor prognosis.[232,233]

#### Proximal Femur

When Ewing's sarcoma occurs in the proximal femur, the femoral head, neck, greater trochanter, and proximal femoral metaphysis will be removed. The resulting defect may be reconstructed with an allograft, proximal femoral replacement prosthesis, or allograft-prosthesis composite. The main determinant of postoperative function is integrity of the abductor mechanism of the hip. [234]

#### Distal Femur

For the older child, adolescent, or young adult, prosthetic reconstruction of the distal femur offers the best functional outcome with the lowest risk of complications. [235] An expandable prosthesis should be used in patients with significant growth potential. [236,237 and 238] In the very young child, van Ness rotationplasty may be used. [239] This operation advances the ankle joint to the level of the knee and functions more like a below-knee than an above-knee amputation.

#### Proximal Tibia

Osteoarticular allograft, proximal tibial prosthesis, allograft-prosthesis composites, and rotationplasty are the available modes of reconstruction in the proximal tibial area.[240] The primary determinant for function of the prosthetics or allografts is success of reconstituting a functional quadriceps mechanism. Soft tissue coverage is also a major consideration in this region. Gastrocnemius muscle flaps often are used to augment coverage of large implants or bone grafts and to provide an additional attachment site for the patellar tendon.

#### Distal Tibia and Foot

As previously noted, the expected function after below-knee amputation is superior to the result of limb-sparing reconstruction. Amputation therefore is preferred over reconstruction in patients with Ewing's sarcoma of the distal lower extremity.

#### Proximal Humerus

The reconstruction options after resection of the proximal humerus are similar to those following resection of the proximal femur: allograft-prosthesis composite, proximal humerus prosthesis, and fusion either to the scapula or clavicle. Achieving effective rotator cuff function after any of these methods is difficult.

#### Distal Humerus

Prosthetic elbow replacement is available for reconstructing the distal humerus. Osteoarticular allograft may also be considered but should not be used in young children with significant growth potential.

#### Scapula

If the glenoid can be spared, resection of the body of the scapula does not result in a major functional deficit. [241] When the glenoid must be removed, fusion of the humerus to the clavicle or leaving the shoulder flail are the available options. Fusion offers the best functional result when it is successful.

#### Fibula, Rib, and Clavicle

The fibula, rib, and clavicle, so-called expendable bones, may be resected with no need for reconstruction. [242]

### Radiotherapeutic Technique

#### *Treatment Planning*

The first step of treatment planning is a careful and complete review of the MRI and CT scans of the primary tumor ( Fig. 33-5). Information regarding the size and extent of the tumor in relation to adjacent normal tissue is essential in designing the treatment field during simulation. MRI has an additional advantage over CT in its ability to show the extent of bone marrow edema (presumably corresponding to intramedullary involvement by tumor) on T2-weighted images. Immobilization or positioning devices such as casts should be used whenever possible to aid in accurately reproducing the treatment position. Biopsy sites and surgical incisions should be marked for simulation so that they can be included in the treatment fields. Addition of contrast medium in bowel or bladder during simulation can help to identify and exclude these organs from the treatment fields. The planning CT scan with the patient in the treatment position usually is very helpful in treatment planning. Recently, CT simulators have become available that combine spiral CT technology with the capability of aligning and marking patients, conducting image reconstruction and computer virtual simulation, and networking to a treatment planning computer. The entire treatment planning process has become more sophisticated. If a planning CT study is not available, contour measurements can be taken at multiple levels to account for changes in dosimetry associated with changes in patient contour.

Complex field arrangements (i.e., wedged pairs, obliques, and rotational fields) can be used during computerized planning to improve dose distribution. Wedge filters and other beam modifiers should be used if necessary. The use of shaped fields with customized Cerrobend blocks usually is recommended to reduce the volume of uninvolved normal tissues in the field. Three-dimensional treatment planning programs now calculate, display, and allow evaluation of volumetric radiation dose distribution to the tumor target and to the surrounding normal tissues. Computer optimization algorithms are increasingly used to facilitate the planning process.

### *Treatment Delivery*

During treatment, weekly port films should be obtained to verify treatment accuracy. If there is a change in patient contour caused by tumor regression, resimulation is required. Close communication between the pediatric and radiation oncologists is important during treatment delivery. Patients should wear soft, loose cotton garments that will not abrade the skin in the radiation portal. Coarse fabric such as denim should be avoided. Careful evaluation of the skin on a regular basis is important.

### *Treatment by Site*

For extremity lesions, a strip of unirradiated tissue should be preserved to prevent the development of edema or fibrosis after circumferential irradiation. A bolus should be used over the biopsy incision or surgical scar unless it is crossed by the radiation beam. Uninvolved epiphyseal plates and joint spaces should be excluded from the radiation field whenever possible. [243] The Achilles tendon, which can undergo late fibrosis after radiation, should be excluded from the treatment volume, if possible. Testicular shielding should be considered for thigh and pelvic lesions. [244] Selected patients with primary lesions of the hand and foot can be treated with radiation and have an excellent functional outcome. [245,246] Sarcomas suitable for this approach are smaller lesions (soft tissue masses less than 3 to 4 cm in diameter) that permit considerable sparing of normal tissue. Because of the continued trauma associated with weight bearing, attempts should be made to spare the sole of the foot. Efforts also should be made to exclude the nail beds from the irradiation fields. Patients with extensive soft tissue involvement that would require circumferential irradiation of the hand or foot or irradiation of a large portion of the sole of the foot and Achilles tendon are not suitable for this approach. Patients presenting with major pretreatment functional deficits related to tumor infiltration of vessels or nerves may also be better served by amputation. Lesions of the calcaneus can be treated unless there is extensive bony destruction or soft tissue distention. There will be moderate loss of heel-pad thickness, but this has been associated with only mild functional impairment. [246]

For pelvic and central axis lesions, special attention to soft tissue extension visualized by MRI is critical. CT treatment planning and complex beam arrangements can minimize the volume of normal tissue (heart, lung, kidney, liver, bladder, bowel, and rectum) in the high-dose region. The buttocks should be taped apart to avoid bolus effect and unacceptable skin reaction. Adequate hydration and use of mesna during cyclophosphamide or ifosfamide administration are advised to minimize the chance of hemorrhagic cystitis, which can be exacerbated by irradiation if the bladder is in the radiation field.

Because of an unexpected finding of central nervous system relapse in the early NCI study, prophylactic cranial irradiation was incorporated in several of the subsequent NCI studies. [216] Central nervous system recurrence without skull bone involvement is now recognized to be extremely rare, and routine use of prophylactic cranial irradiation is unnecessary. [187]

In the IESS-1 study, prophylactic whole-lung irradiation (15 to 18 Gy) was used in one of the treatment arms. Although this treatment decreased subsequent pulmonary recurrence, significant pulmonary toxicity occurred when such irradiation was combined with multi-agent chemotherapy. In addition, doxorubicin [Adriamycin (Adr)], as a component of multi-agent chemotherapy, was shown to reduce recurrences in both the lungs and the bones. On the other hand, a German study in patients with pulmonary metastatic disease at diagnosis showed improved survival for those who received 18 to 21 Gy as compared to those receiving 12 to 14 Gy or no irradiation.

### *Treatment Volume*

The traditional wisdom is to include the entire medullary cavity for at least part of the treatment and a 3- to 5-cm margin beyond the soft tissue extension. In IESS-1, an increase in local recurrence was noted with treatment fields that did not include the opposite epiphysis. However, three studies in the 1980s have shown that less than whole-bone radiotherapy can be as effective as whole-bone irradiation for lesions smaller than 8 or 9 cm in diameter. In the St. Jude Children's Research Hospital study, local control was achieved in this manner in 12 of 14 patients with lesions smaller than 9 cm. [247] Investigators in the University of Florida also reported excellent local control using tailored radiation fields, although in patients with tumors larger than 8 cm, total body irradiation (TBI) was added, thus delivering some radiation to the entire bone. [216] The POG prospectively tested whole-bone versus tailored irradiation fields (2-cm margin around the pre-chemotherapy tumor). After the preliminary analysis showed that limited treatment volume was as effective as whole-bone irradiation, the randomized study was modified to a single-arm study using only tailored fields. [167,248]

Currently, tailored radiotherapy fields are recommended whenever possible. The initial treatment volume for patients should include the entire bony lesion, as demonstrated by initial imaging studies, with a 2-cm margin. If a 2-cm margin necessitates irradiation of the epiphysis of an adjacent bone and there is no extension across the joint space, a smaller margin should be used to include the adjacent epiphysis. Whether the initial soft tissue extension plus a 2-cm margin should be irradiated is subject to debate. Depending on the size of the initial soft tissue mass, the volume of irradiation could be substantial if the entire volume is to be covered: For example, the volume could involve the entire circumference of an extremity or the entire pelvis. In the pelvis, at least, most experts would agree to use the postinduction soft tissue volume for radiation treatment planning, to reduce bowel or bladder toxicity. In any case, if the volume of tumor (bone and soft tissue) at diagnosis is included in the initial radiation treatment volume, the boost fields could certainly allow a field reduction that takes into consideration the response of the soft tissue component to chemotherapy. If the initial soft tissue mass extends into a body cavity such as the pelvis or lung, the recommended treatment volume includes a 2-cm margin around the post–induction chemotherapy soft tissue volume, so as to minimize normal tissue morbidity.

Because the bony component of the tumor seldom shows a change in dimension on CT or MRI scans after induction chemotherapy, no field reduction for the bony component is possible in most cases. If gross residual disease is noted after resection, the initial postoperative radiotherapy volume should be the initial bony and soft tissue volume plus a 2-cm margin. After this, the site of gross residual disease should receive a boost treatment with a 2-cm margin. The presence of any microscopic residual disease (e.g., positive or inadequate surgical margin) after resection dictates that the postoperative radiotherapy volume be similar to that described for gross residual disease. A boost treatment with a 2-cm margin around the site of microscopic disease (if known) should be considered.

TBI or sequential hemibody irradiation as part of systemic treatment for patients with high-risk features or metastatic disease has been investigated in a limited fashion in the Princess Margaret Hospital, [249,250] NCI, [251] and Universityof Florida studies. [252] TBI has not contributed significantly to the control of metastatic disease, although definitive conclusions are difficult to ascertain from these single-arm studies.

### *Dose*

In the IESS-1 study, no clear dose-response relation was noted in the 40- to 60-Gy range. With few local recurrences and 50% of patients dying from distant disease within 3 years of entry into the study (perhaps before they survived long enough to manifest a local recurrence), correlating local control with total dose was difficult. An analysis of control of pelvic lesions also did not demonstrate any dose-response relation in the 40- to 60-Gy range. [253] Data from Massachusetts General Hospital showed no difference between doses of less than 60 Gy and doses of more than 60 Gy in providing local control of lesions measuring less than 1,000 cc. [213] Patients with localized extremity Ewing's sarcoma on IESS-1 were randomly assigned to receive total radiation doses of 46 or 60 Gy. Local recurrences were seen in five of nine and five of eight patients, respectively, at these two dose levels. Because of the inadequacy of the radiotherapy volume in the initial portion of this study (as described previously), a meaningful conclusion regarding dose response is not possible.

At the Instituto Rizzoli, investigators noted a 41% local recurrence rate at doses of 40 to 55 Gy, as compared with 30% at doses of 56 to 60 Gy in patients whose primary tumors were treated with irradiation alone. [198]

Despite lack of definitive data, a common pattern of practice has emerged: Where whole-bone irradiation is performed, 40 to 50 Gy is delivered to the entire medullary cavity, followed by a boost of 10 to 15 Gy to the primary tumor, resulting in a total tumor dose of 55 to 60 Gy.

Lower doses (30 to 36 Gy; mean, 35 Gy) have been investigated at the St. Jude Children's Research Hospital. [247] For small lesions (less than 8 cm) and a favorable response to induction chemotherapy, local control was 90%. However, local control was inferior for lesions larger than 8 cm (52%; $p = .054$). Further studies are needed before lower doses of radiation can be accepted as standard practice.

Currently, use of tailored fields with a dose of 45.0 Gy (at 1.8 Gy per fraction) is recommended. An additional 10.8 Gy should be delivered to the boost volume. The total tumor dose is 55.8 Gy. In patients in whom the soft tissue mass shows less than 50% regression after induction chemotherapy, a surgical resection or a final

tumor dose of 60 Gy (or both) should be considered.

Patients with gross residual disease after surgery should receive 45 Gy to the initial treatment volume, as described previously, with a boost of 10.8 Gy to areas of residual disease. For postoperative treatment of microscopic disease, 45 Gy to the initial tumor volume currently is recommended. A boost of 5 Gy to the site of microscopic disease, if known, is advisable.

***Schedule***

A conventional schedule of radiotherapy is 1.8 to 2.0 Gy per fraction, once daily, 5 days per week. A hyperfractionated schedule of 1.2 Gy twice daily, 6 hours apart, has been tested in three protocols at the University of Florida. [252] Those patients who did not have a soft tissue mass at diagnosis or whose soft tissue mass completely responded after induction chemotherapy received 50.4 Gy; those who experienced 50% or greater resolution of the soft tissue mass received 55.2 Gy; and those in whom less than 50% regression of the soft tissue mass was demonstrated or who experienced progressive disease during induction chemotherapy received 60 Gy. All patients with high-risk features also received TBI. The 5-year local control rate ranged from 80% to 92% in these protocols. In patients evaluated for limb function, the late effects were reported to be minimal and no treatment-related pathologic fractures were noted. These results indicate that hyperfractionated radiotherapy with small treatment fraction size can potentially reduce late morbidity without sacrificing local tumor control. However, this approach must be studied in a larger group before it can be recommended.

In the CESS-86 study, an accelerated fractionation schedule of 1.6 Gy twice daily to a total of 60 Gy was used. The final analysis showed no differences in local control between this schedule and the conventional once-daily radiotherapy schedule. [254] In irradiated patients, hyperfractionated split-course irradiation and conventional fractionation yielded the same results: 5-year overall survival of definitively irradiated patients, 63% after conventional fractionation and 65% after hyperfractionation; relapse-free survival, 53% versus 58%; and local control, 82% versus 86% (not significant). [254] On the basis of this study, it can be hypothesized that accelerated clonogenic repopulation between treatment fractions and overall treatment time may not be important factors in the local control of Ewing's sarcoma.

Single large-fraction (10- to 20-Gy) intraoperative radiotherapy has been studied in a limited number of patients. The NCI group reported use of intraoperative radiation (20 Gy) in two patients with large pelvic sarcomas that had completely regressed and were positive on biopsy after induction chemotherapy and 60-Gy external-beam irradiation; the lesions converted to completely responsive after intraoperative irradiation. [255] Calvo et al.[256] reported the use of induction chemotherapy plus external-beam irradiation (40 to 50 Gy), followed by partial or total surgical resection plus intraoperative irradiation of 10 to 15 Gy, in eight previously treated patients and re-induction chemotherapy plus 20-Gy intraoperative irradiation in three patients with local recurrence. With a median follow-up time of 18 months, local control was achieved in 10 of 11 patients. Whether this approach truly offers benefit in patients with large lesions that fail to regress with initial induction chemotherapy and whether the incidence and severity of late morbidity are increased require further study.

***New Radiation Approaches***

Three-dimensional treatment planning computer programs with beam's-eye view capability allow for even more complex design of multiple coplanar or non-coplanar treatment fields that can more effectively reduce the radiation dose to critical adjacent normal structures. These computer programs are particularly useful in planning the treatment of central lesions (e.g., in the pelvis or trunk). Multileaf collimators that can be programmed to shape the treatment fields obviate the need for Cerrobend blocks, and their use can be incorporated into many of the three-dimensional treatment planning programs. Generically, this form of sophisticated radiotherapy is called *conformal radiotherapy*, which consists of precision treatment designs that conform the spatial distribution of high radiation doses to the target volume while concomitantly excluding as much of the surrounding normal tissue as possible.

Intensity-modulated radiotherapy is the latest technology for conformal radiation planning and delivery. [257] Multiple small radiation beams of differing intensities are combined to create the dose envelope around the target. Because of the large number of iterations needed to find the right combination of these beamlets, one form or another of computer optimization algorithms generally is required in the planning process. A specialized intensity-modulated radiotherapy system combining such features as spinal CT and an intensity-modulated multivane collimator is being constructed, and clinical testing will begin in the near future.

A unique conformal radiotherapy system called the *Peacock* now is undergoing clinical investigation. [258] This system uses dynamic radiation intensity modulation and computer optimization. *Dynamic intensity modulation* means varying the amount of radiation delivered in a continuous fashion as the linear accelerator rotates around the patient. A special multivane collimator can generate a combination of $3.6 \times 10^{22}$ dose intensity patterns. The computer program finds the solution by choosing the appropriate dose intensity patterns needed to create the desired dose envelope that conforms to the shape of the target. Conceptually, this process is similar to putting together a three-dimensional jigsaw puzzle using a variety of small pieces of different sizes, each representing a different amount of radiation. Clinical situations exist in which conventional radiotherapeutic technique could not avoid significant morbidity if the tumor were to be adequately irradiated. The Peacock system could generate a better treatment plan. For example, in Ewing's sarcoma in the posterior aspect of a rib, conventional radiotherapy would irradiate a large volume of lung as well as the breast, but the conformal techniques such as Peacock allow significant lung sparing ( Fig. 33-10). Increasing use of such advanced systems is anticipated and will provide better radiotherapy for Ewing's sarcomas, especially those involving the trunk and pelvis.

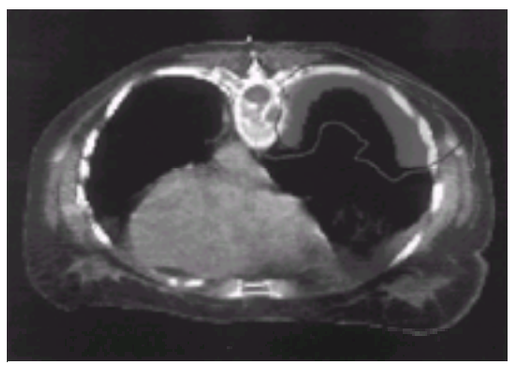


**FIGURE 33-10.** A Peacock treatment plan for a posterior rib tumor on an axial computed tomographic image. The 75% isodose wraps around the target. The 45% isodose curves avoid the spinal cord and generally spare the heart and anterior left lung.

**Chemotherapy**

Before the routine use of adjuvant chemotherapy for the treatment of the ESFT, only 10% of patients survived 5 years. [205,206,259,260] In the early 1960s, cyclophosphamide (C), actinomycin-D (A), and vincristine (V) were found to be active against Ewing's sarcoma. By the late 1960s, patients were treated with these drugs as adjuvants to local therapy at the NCI, St. Jude Children's Research Hospital, and a few other institutions. [261,262,263 and 264] A number of patients so treated remained free of disease for more than 2 years, a previously rare event. The investigators involved in these cases recognized that chemotherapy was having an impact on the disease process, and the subsequent studies were expanded. It was demonstrated that doxorubicin (D) was also active against Ewing's sarcoma of bone, and this drug was incorporated into the adjuvant regimens. Long-term follow-up from the early adjuvant trials at the NCI demonstrates that Ewing's sarcoma of bone can be cured, with 33% of patients surviving free of disease for 15 years after treatment onset. [176,265] Clinical trials during the last two decades have focused on identifying more effective drug regimens.

Table 33-6 lists the activity of drugs against the ESFT as determined by phase II studies. Classic alkylating agents such as cyclophosphamide, ifosfamide, and high-dose melphalan are among the most active agents. Recent phase II studies involving these diseases have been carried out in patients with tumors resistant to intensive multi-agent regimens. For example, cisplatin, topotecan, and thiotepa have been found to be inactive against refractory or recurrent Ewing's sarcoma. [266,267 and 268] Cisplatin, which has been determined to be active against osteosarcoma, rhabdomyosarcoma, and neuroblastoma, showed little activity against drug-resistant Ewing's sarcoma of bone.[268] However, cisplatin might be active in a newly diagnosed case of the ESFT. Likewise, the demonstration of a high level of activity for the drug combination ifosfamide and etoposide in patients with recurrent disease classified among the ESFT was impressive [269] and led to the use of this combination in

newly diagnosed cases.[270,271] and [272] Meyer et al.[272] at St. Jude treated with ifosfamide and etoposide (before other agents) 26 patients with newly diagnosed Ewing's sarcoma and PPNET, to determine the rate of response. They found that 25 of 26 evaluable patients demonstrated at least a partial response. [272] No additional drugs have been recognized to be active against the ESFT in the last several years.

| Agent and studies | Response (no. responding/total) | Response rate (%)[a] |
|---|---|---|
| Cyclophosphamide[329-333] | 17/36 | 47 |
| Doxorubicin[334-340] | 25/60 | 42 |
| Ifosfamide[341-343] | 12/37 | 32 |
| Vincristine[332,344-346] | 4/10 | 40 |
| Actinomycin-D[331,342,348] | 3/16 | 19 |
| BCNU[331,349] | 6/18 | 33 |
| 5-Fluorouracil[350,351] | 4/10 | 40 |
| 5-Fluorouracil/leucovorin[352] | 0/12 | 0 |
| Etoposide[353,354] | 3/10 | 30 |
| Cisplatin[308,355-357] | 2/27 | 7 |
| Carboplatin[358] | 0/7 | 0 |
| Melphalan (high-dose)[359,360] | 9/11 | 82 |
| Ifosfamide/etoposide[355] | 18/30 | 60 |
| Topotecan[361] | 1/26 | 4 |
| Thiotepa[356] | 0/10 | 0 |

BCNU, bischloroethylnitrosourea (carmustine).
[a]Complete response or partial response.

**TABLE 33-6. SINGLE-AGENT AND COMBINATION ACTIVITY IN EWING'S SARCOMA IN PHASE II STUDIES**

### *Combination Chemotherapy Studies*

The major combination chemotherapy studies are summarized in Table 33-7. One of the first such studies of Ewing's sarcoma of bone was carried out at the NCI. Patients were treated with VAC with or without doxorubicin and concomitant local irradiation. [176] These chemotherapeutic regimens were of relatively low intensity when judged by today's standards. Nonetheless, one-third of the patients were long-term survivors. Results were best in patients with nonmetastatic extremity tumors.[189]

**TABLE 33-7. OUTCOME OF MAJOR MULTIMODALITY STUDIES**

### Nonmetastatic Disease

In 1973, the first IESS study (IESS-1) was initiated to compare results of three adjuvant treatment regimens for nonmetastatic Ewing's sarcoma of bone. [183] This national study was a collaboration by three multi-institution groups: the Children's Cancer Study Group (CCSG), the Southwest Oncology Group, and the Cancer and Acute Leukemia Group B. Because the initial goal was to test the value of adjuvant chemotherapy, one-third of the patients were to be randomized to receive no chemotherapy. After two of the first three patients who did not receive chemotherapy experienced a relapse, the study was amended to include three groups: VAC (vincristine, actinomycin-D and cyclophosphamide) alone, VAC plus Doxorubicin (D) or VAC plus bilateral pulmonary irradiation. [183]

The results of this study have been updated with a median follow-up time of 6 years for surviving patients ( Fig. 33-11).[208] VAC plus D was more effective than VAC plus pulmonary irradiation, which in turn was superior to VAC alone, with 5-year relapse-free survival rates of 60%, 44% and 24% respectively. The majority of failures was attributable to metastatic disease, which occurred in 44% of the patients, whereas 15% of failures was attributable to local disease. The frequency of lung metastases was the same for those receiving VAC alone and those receiving VAC plus pulmonary irradiation.

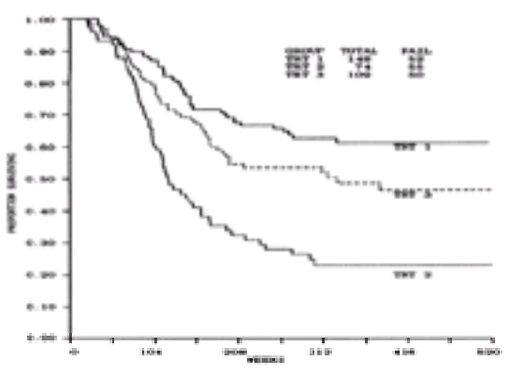


**FIGURE 33-11.** Actuarial survival of patients treated on Intergroup Ewing's Sarcoma Study 1. Treatment 1 (TRT1) consists of vincristine, actinomycin-D, and cyclophosphamide (VAC) plus doxorubicin; TRT2 is VAC plus lung irradiation; TRT3 is VAC alone.

Having established a role for doxorubicin in the treatment of Ewing's sarcoma of bone, a second IESS study (IESS-2) was launched in 1978 to compare the efficacy of high-dose intermittent versus moderate-dose continuous VAC plus D in patients with localized, nonpelvic Ewing's sarcoma of bone. [273] The major difference between the two regimens was a 150% increase in doxorubicin dose intensity during the initial 36 weeks of therapy. The relapse-free survival rate at 5 years was significantly higher among patients treated with the high-dose intermittent regimen (73% vs. 56%; $p = .03$) thus indicating that increasing doxorubicin dose intensity and administering it early in the course of therapy is beneficial. As in IESS-1, the majority of treatment failures was metastatic.

Investigators at St. Jude developed an alternative VDCA (vincristine, doxorubicin, cyclophosphamide and actinomycin-D) regimen that included induction with oral cyclophosphamide daily for 7 days, followed by intravenous doxorubicin on day 8. This well-tolerated regimen was delivered every three weeks. After 3 months, a second-look surgical procedure was performed, and radiotherapy was administered. [275] The excellent response and progression-free survival rates achieved with this regimen prompted the POG to use it in a protocol that included a randomized radiotherapy question. [248] This study achieved a 3-year EFS of 54% for patients with localized tumor; the majority of the failures was systemic. These disappointing results led the POG to abandon this regimen in favor of high-dose intermittent VDCA in the subsequent study.

Most recently, two strategies for improving the outcome of patients with localized ESFT have been tested. The first strategy was tested in two historically controlled

studies which investigated the efficacy of substituting ifosfamide (Ifos) for cyclophosphamide in the VDCA regimen. The German Pediatric Oncology Group's CESS-86 study reported a minimally improved 3-year DFS rate with ifosfamide, whereas the French Society of Pediatric Oncology reported no benefit. [210,276] In European Intergroup Cooperative Ewing's sarcoma study 92, which is a project of the German and British societies for pediatric oncology, 470 patients with localized ESFT were stratified into two treatment groups based on tumor volume (less than and at least 100 mL); standard-risk or high risk categories. Standard-risk patients were randomized to cyclophosphamide-versus ifosfamide-based maintenance after induction with VDIfosA (vincristine, doxorubicin, ifosfamide, actinomycin-D). The EFS at 5 years for the standard-risk patients was 75%. In regard to the randomization, the 5-year EFS was 71% for VDCA and 79% for VDIfosA ( $p = .37$). High-risk patients were randomized to VDIfosA versus the addition of etoposide. The EFS at 5 years for the high-risk patients was 58%, and the randomization results were 54% for VDIfosA and 62% for etoposide, VDIfosA ($p = .60$).[280] The substitutionof ifosfamide for cyclophosphamide did not improve on outcome.

The second strategy examined more recently has been whether the addition of ifosfamide and etoposide to the VDCA regimen improves survival. The phase II results that demonstrated a high degree of activity of ifosfamide and etoposide in patients with recurrent ESFT led to studies at the NCI and St. Jude that integrated these agents into VDCA regimens for patients with newly diagnosed disease.[270,272]

A phase III POG-CCG intergroup clinical trial (IESS-3) completed in 1992, compared the efficacy of VDCA with and without ifosfamide and etoposide in ESFT. [277] The study accrued 398 nonmetastatic patients (median age, 12 years; range, 0 to 28 years); 188 extremity tumors and 93 pelvic tumors were included. The 5-year EFS rate for nonmetastatic patients was 54% for VDCA and 68% for VDCA, ifosfamide, and etoposide ($p = .0042$) (Fig. 33-12). Overall survival rates were 61% and 72%, respectively ($p = .01$). For the entire group, failures were local in 27%, systemic in 63%, and combined in 10%. This study substantiates the benefit of the addition of ifosfamide and etoposide for patients with localized ESFT.

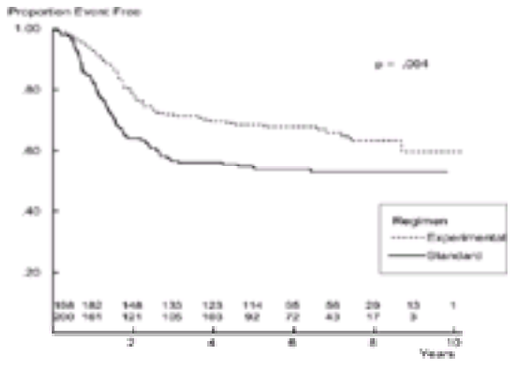



**FIGURE 33-12.** Results of Intergroup Ewing's Sarcoma Study 3. Event-free survival comparing outcome by regimen. Standard arm represents those patients receiving vincristine, doxorubicin, cyclophosphamide, and actinomycin-D (VDCA). Patients on the experimental arm received alternate courses of VDCA and ifosfamide and etoposide used in combination.

The Orthopedic Institute from Rizzoli conducted two sequential studies in which the addition of ifosfamide and etoposide to the VDCA regimen in the neoadjuvant treatment of ESFT seemed to confer no advantages.[281] The first study (REN-1) involved 108 patients with ESFT between 1983 and 1988 and used VDCA. The second study (REN-2) involved 82 patients with ESFT treated between 1988 and 1991. The chemotherapy used was VDCA and ifosfamide and etoposide. No advantage was observed when ifosfamide and etoposide were added to the VDCA regimen. The 5-year DFS and overall survival in REN-2 were 54% and 59% respectively, and in REN-1 were 50% and 56%, respectively. Because these two studies were performed in the same institute, the choice of local treatment followed the same criteria. Additionally, the distribution of patient characteristics (i.e., tumor site, tumor volume, and serum LDH values) was similar in the two studies. Although the results of REN-1 and REN-2 appear on first glance to contradict IESS-3, there are a number of reasons for the different results. First, more cycles of the combination ifosfamide and etoposide were given in IESS-3 than in REN-2 (ten cycles vs. three cycles); second, in IESS-3, the ifosfamide-etoposide combination was initiated during induction, whereas in REN-2, ifosfamide and etoposide therapy was begun during the maintenance phase. [277] The additionof ifosfamide and etoposide to the backbone of V, D, and C has become the current standard of care for patients with localized ESFT.

Because ESFT are very sensitive to alkylating agents, which have a steep dose-response curve, the use of dose intensification to improve DFS in patients with ESFT is of much interest. Increasing the dose intensity means increasing the quantity of a drug per unit of time. Granulocyte colony-stimulating factor (G-CSF) has made the dose-intensive protocols possible by reducing the duration of neutropenia, which is the most dangerous side effect of dose-intensive therapy. St. Jude Hospital investigators recently published their experience with dose intensification for pediatric patients with ESFT. [282] From 1992 to 1996, St. Jude treated 51 patients with ESFT with their dose-intensified Ewing's protocol, in which the dose intensity was 1.5 (minimum increase in ifosfamide) to 2.5 times (maximum increase for cyclophosphamide) higher than that delivered on the St. Jude protocol in the prior Ewing's sarcoma study. [272] Dose intensity was achieved by increasing the total doses of drugs administered. Toxicity was considerable in this study, particularly infectious complications. Fourteen patients developed sixteen episodes of bacteremia, six of which were associated with septic shock. From this group of patients, it appeared that dose intensification was feasible during induction, with very few delays in therapy, but that, in the maintenance phase after radiotherapy had been given, dose intensification was feasible for only a small percentage of patients. Estimated 3-year survival and EFS were 72% and 60%, respectively. The authors conclude that because the toxicity of dose intensification is high, it will be important to first determine its impact on DFS before deciding whether to place patients on these toxic regimens.

A POG-CCG Ewing's sarcoma study recently closed, which compared a 48-week standard regimen of VDC alternating with ifosfamide and etoposide with G-CSF with a 30-week dose-intensified regimen of the same chemotherapeutic agents. The experimental regimen had fewer cycles with higher doses of cyclophosphamide and ifosfamide, so that the total drug doses in the two regimens were the same. Preliminary data indicate that there is no difference in the EFS of patients treated over 48 weeks versus 30 weeks.

A prospective pilot study from the Children's Hospitals of Philadelphia and Seattle achieved dose intensification through interval compression (decreasing intervals between cycles while maintaining conventional doses). [283] Interval compression allows dose intensification of all chemotherapeutic agents, not just the alkylators, and it limits the time in which partially drug-resistant cells have to recover from one cycle of chemotherapy before they are attacked by the subsequent cycle. This pilot study included 73 children and adolescents with Ewing's sarcoma, rhabdomyosarcoma, and miscellaneous soft tissue sarcomas. [283] The data demonstrated that compression of the interval between chemotherapy cycles using G-CSF is a well tolerated and practical method of increasing the dose intensity of chemotherapy. In the induction phase, the median chemotherapy cycle interval was 16 days, with a median average relative dose intensification of 1.27 as compared with every-21-days therapy. Although the primary objective of this study was practicality, some survival information was also gleaned. Of the 73 patients, 30 had localized ESFT and two-thirds of these patients were at relatively high risk secondary to axial primary lesions. For the 30 patients with localized ESFT, the EFS was 73%, and the overall survival was 85% at a median follow-up of 30 months.283 This regimen is not less effective than previous protocols.

The Children's Oncology Group (COG) has begun a Ewing's sarcoma study for patients with localized disease. The study is a randomized controlled trial comparing interval compression with standard every-3-weeks chemotherapy. In the COG study, the objective will be to evaluate the effect of interval compression with G-CSF support on the EFS and overall survival of patients with ESFT.

### Metastatic Disease

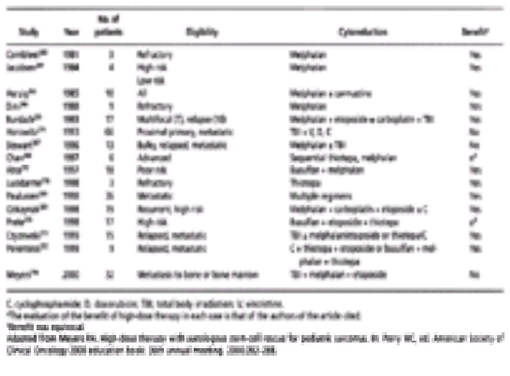
Results of the treatment of patients with clinically overt metastatic disease at diagnosis are significantly less favorable than are the results for patients with localized disease (Table 33-7). For example, only 2 of 27 patients with metastatic Ewing's sarcoma of bone who were treated at the NCI from 1968 to 1980 were surviving 5 years after diagnosis, as opposed to 41% of patients with localized disease ( $p < .0001$).[176] An intergroup study for patients with metastatic Ewing's sarcoma of bone, conducted from 1975 to 1977, achieved complete responses in 31 of 44 evaluable patients with a regimen that included VAC plus D and irradiation of the primary site and a maximum of four metastatic sites.[175] If lung metastases were present, bilateral pulmonary irradiation was administered. In both studies, approximately 30% of patients were recurrence-free at 3 years after diagnosis. Age was a significant prognostic factor: More than 40% of those younger than 11 years at treatment were alive 10 years later in contrast to fewer than 20% of the older children. A higher proportion of the younger children had rib primary lesions and pleural effusions as the sole site of metastasis, which is a more favorable group. The best results reported for metastatic Ewing's sarcoma of bone were achieved with a sequential C plus D induction regimen administered by the St. Jude Children's Research Hospital. [284] The researchers reported a favorable DFS rate of 55% at 47 months for 18 patients

with metastases.[174] This protocol was used subsequently in the multicenter French Society of Pediatric Oncology (SFOP) trial EW88; however, a DFS of only 21% was reached at 3 years.[285,286] Finally, the addition of ifosfamide and etoposide has not improved the outcome for patients with metastatic disease at diagnosis. [277,278]

Many groups have supplemented chemotherapy with irradiation to sites of gross metastatic disease in addition to the primary site. [178,185,287] Patients with solitary or circumscribed bony lesions can be irradiated at those sites to doses in the range of 40 to 50 Gy, in addition to irradiation of the primary tumor. Low-dose (15 to 18 Gy in 150-cGy fractions), bilateral pulmonary irradiation in conjunction with intensive chemotherapy has been reported to be efficacious in controlling gross metastatic disease in the lungs without significant pulmonary toxicity. [287] Additional small, field boost doses to a total of 40 to 50 Gy can be provided to large, focal lung lesions.

Discouraging results with conventional chemotherapy and irradiation for metastatic ESFT have led to the consideration of novel approaches. Because the ESFT are relatively radiation-sensitive, the use of radiotherapy as a systemic treatment for the ESFT has long been of interest. [249,288,289] Initial approaches entailed the use of sequential hemibody irradiation; recent studies have used TBI as a systemic agent in conjunction with chemotherapy and bone marrow transplantation as a rescue. A protocol conducted at the NCI from 1983 to 1986 treated patients who had high-risk Ewing's sarcoma of bone with high-dose VDC, irradiation to local and metastatic sites, and intensification with a final cycle of VDC and TBI (800 cGy over 2 days), followed by autologous bone marrow re-infusion. [178] Although preliminary results were encouraging, therapy has failed in more than 80% of patients with metastatic Ewing's sarcoma and PPNET.[279,290]

The potential therapeutic benefit of very-high-dose chemotherapy regimens in combination with stem-cell rescue for patients who have metastatic disease, refractory or relapsed disease, or large axial inoperable tumors has been under evaluation by multiple centers worldwide ( Table 33-8). Comparing these studies is difficult because they do not share common eligibility criteria. Across the series of studies that have been completed, no consistent definition of what constitutes a high-risk Ewing's sarcoma patient appears. This point is particularly important, because patients with pulmonary metastases reportedly have a somewhat improved prognosis over patients with bone and/or bone marrow metastases.[299,300] Additionally, different regimens for cytoreduction have been used, as have various sources for stem cells. This makes very difficult the interpretation of the data and the drawing of any sound conclusions of the benefit of very-high-dose chemotherapy in combination with stem-cell rescue. Burdach et al.[291] treated 17 patients who had multifocal Ewing's sarcoma or who had experienced relapse within 2 years of diagnosis with TBI (1,200 cGy hyperfractionated) and myeloablative chemotherapy (melphalan, 30 to 45 mg per $m^2$ per day on 4 consecutive days followed by one dose of etoposide 40 to 60 mg per $m^2$). The relapse-free survival rate was 45% ± 12% at 6 years after the last event before transplant. [291] Atra et al.[292] studied 18 patients with poor-risk disease classified within the ESFT who received consolidation therapy with busulfan and melphalan. After a median follow-up of 2 years, 13 surviving patients remained in complete remission. Lucidarme et al.[293] reported on a phase II study of high-dose thiotepa and autologous rescue in three patients with refractory metastatic Ewing's sarcoma, two of whom went into partial remission and one of whom experienced progressive disease. One patient is still alive more than 28 months after re-infusion.[293]



| Study | Year | No. of patients | Eligibility | Cytoreduction | Benefit* |
|---|---|---|---|---|---|
| Cornbleet[294] | 1991 | 3 | Refractory | Melphalan | Yes |
| Jacobsen[295] | 1994 | 4 | High risk<br>Low risk | Melphalan | Yes |
| Ara et al[296] | 1995 | 10 | All | Melphalan ± permutine | Yes |
| Dini[297] | 1998 | 5 | Refractory | Melphalan | Yes |
| Burdach[291] | 1993 | 17 | Multifocal (?), relapse (10) | Melphalan + etoposide ± carboplatin + TBI | Yes |
| Horowitz[178] | 1993 | 66 | Proximal primary, metastatic | TBI + C, D, E | No |
| Stewart[287] | 1996 | 13 | Bulky, relapsed, metastatic | Melphalan ± TBI | No |
| Chan[298] | 1997 | 6 | Advanced | Sequential therapy, melphalan | n† |
| Atra[292] | 1997 | 18 | Poor risk | Busulfan + melphalan | Yes |
| Lucidarme[293] | 1998 | 3 | Refractory | Thiotepa | Yes |
| Thadani[299] | 1998 | 26 | Metastatic | Multiple regimens | Yes |
| Cohn-razal[300] | 1998 | 15 | Recurrent, high risk | Melphalan + carboplatin + etoposide ± C | Yes |
| Peter[301] | 1998 | 17 | High risk | Busulfan + etoposide + thiotepa | n† |
| Claviez[302] | 1999 | 15 | Relapsed, metastatic | TBI ± melphalan/etoposide or thiotepa/C | Yes |
| Penarrubia[303] | 1999 | 9 | Relapsed, metastatic | C + thiotepa + etoposide or busulfan + melphalan + thiotepa | Yes |
| Meyers[304] | 2000 | 32 | Metastasis to bone or bone marrow | TBI + melphalan + etoposide | No |

C, cyclophosphamide; D, doxorubicin; TBI, total-body irradiation; V, vincristine.
*The evaluation of the benefit of high-dose therapy in each case is that of the author of the article cited.
†Benefit was equivocal.
Adapted from Meyers PA: High-dose therapy with autologous stem-cell rescue for pediatric sarcomas. In: Perry MC, ed: American Society of Clinical Oncology 2000 education book. 36th annual meeting. 2000:262–268.

**TABLE 33-8. HIGH-DOSE THERAPY WITH AUTOLOGOUS STEM-CELL RESCUE FOR HIGH-RISK EWING'S SARCOMA**

The CCG recently completed a prospective trial of high-dose chemotherapy and radiotherapy as consolidation for patients with high-risk Ewing's sarcoma. [294] In this study, the high-risk group included patients who presented with primary Ewing's sarcoma of bone and metastases to bone marrow or to bony sites distant from the primary tumor. Thirty-two patients were eligible and entered into the study. These 32 patients were given induction therapy with three cycles of VDC, which were alternated with two cycles of ifosfamide and etoposide. Local control included surgery for selected patients and radiotherapy to the primary tumor and selected sites of metastases. The patients then received TBI in 3 days, followed by melphalan and etoposide over 3 days, followed by peripheral blood stem-cell re-infusion. Of the cohort of 32 patients, 8 did not receive high-dose therapy because of incomplete response, progressive disease, or toxic death. Twenty-four received TBI, melphalan, etoposide, and stem-cell re-infusion. The EFS for the cohort of 32 patients was 16% at 2 years after diagnosis, which was similar to the EFS achieved in the national trial of conventional chemotherapy and radiotherapy. [295] Induction chemotherapy followed by melphalan, etoposide, TBI, and peripheral blood stem-cell support did not improve prognosis for patients with newly diagnosed high-risk Ewing's sarcoma.

The results from the European Bone Marrow Transplant Solid Tumor Registry were similarly disappointing in regard to patients with metastatic ESFT. Patients in their first complete remission but who had metastases at diagnosis (32 patients) achieved a 5-year survival rate of 21%. [286]

Currently, no conclusive evidence supports the contention that high-dose therapy for patients at high risk of failure is beneficial. To date, the myeloablative regimens used have been too varied to permit meaningful comment. More homogeneous therapy is needed if we are to accumulate sufficient patients for randomized studies in this subject.

### *Treatment of Peripheral Primitive Neuroectodermal Tumor*

PPNET responds to the same chemotherapeutic agents as are used in the treatment of Ewing's sarcoma of bone. The German Society of Pediatric Oncology retrospectively reviewed their experience with 42 patients having this tumor, most of whom were treated on their cooperative group protocols for Ewing's sarcoma with VAC plus D or V-ifosfamide-C plus D.[181] Sixty-three percent of the 32 patients with localized disease were in continuous complete remission, whereas treatment failed in nine of ten patients presenting with metastatic disease; these results are similar to those for patients with Ewing's sarcoma on the same protocol.

At the NCI, 17 patients with PPNET were treated on the same protocol as were those with Ewing's sarcoma, which included an intensive VDC regimen, local irradiation, and TBI (800 cGy) for patients with metastatic disease at diagnosis or high-risk primary sites such as the central axis. [177] Just as for those with Ewing's sarcoma, approximately one-half of the patients with localized disease remain in continuous complete remission, whereas more than 90% of those with metastatic disease at diagnosis have experienced disease recurrence.[290]

At St. Jude Children's Research Hospital, investigators retrospectively reviewed 14 patients with PPNET diagnosed between February 1988 and January 1992. [296] These patients all were enrolled in the “Ewing's 87” (EWI-87) protocol at the hospital. Of the 14 patients, three had metastatic disease and 11 had localized disease. Of the patients with local disease, eight demonstrated no evidence of disease at a median of 59 months, and one of the three with metastatic disease also exhibits no evidence of disease. This study shows that Ewing's sarcoma–directed multimodality therapy can cure approximately two-thirds of patients with PPNET. This figure is comparable to the outcome of patients with EES treated on rhabdomyosarcoma protocols and to that of patients with osseous Ewing's sarcoma. No data are available to suggest that PPNET should be treated differently from Ewing's sarcoma.

### *Treatment of Extraosseous Ewing's Sarcoma*

EES has, until recently, been treated on protocols for rhabdomyosarcoma (RMS). In the Intergroup Rhabdomyosarcoma Studies (IRS), the basic local control strategy was an attempted wide local excision of the primary site, to be followed by postoperative irradiation in the case of a positive margin, positive nodes, or gross residual disease. One hundred thirty patients with EES were registered on three intergroup RMS clinical trials (IRS-I, -II, and -III) from 1972 to 1991. One hundred fourteen of these patients had localized disease. All patients were given multi-agent chemotherapy, and most received irradiation. One hundred seven (82%) achieved complete remission. At 10 years, 62%, 61%, and 77% of the patients were alive after treatment on IRS-I, IRS-II, or IRS-III, respectively. This is similar in percentage to all IRS

patients.[297] This series indicates that EES in children is similar to RMS in its response to multimodality therapy.

Current POG-CCG Ewing's sarcoma studies accept these patients as well. St. Jude placed three children with localized EES (primary sites: chest wall, back, and pelvis) on EWI-87 study and all three had no evidence of disease after a median follow-up of 77 months. [296] Ahmad et al.[298] recently reported on 24 patients with EES. The average length of follow-up of survivors was 64 months. This study included five patients with metastatic disease at diagnosis. With multimodal therapy, the overall 5-year survival rate was 61%, and the DFS rate was 54%. The results of these studies demonstrate that EES should be treated with multimodality therapy and that the results are similar to those in patients with osseous Ewing's sarcoma.

### *General Guidelines for Treatment*

Patients with the ESFT should, if possible, be treated on protocols specifically designed for these diseases. If such clinical trials are not available, therapy should be guided by the following principles: First, the patient should be evaluated as thoroughly as possible, as described in this chapter, to establish an accurate pretreatment baseline. Second, that baseline should guide local therapy, which should be administered under the guidance of physicians with special expertise in these diseases. Third, systemic chemotherapy should include VDC and, for those patients with localized tumors, ifosfamide and etoposide. The specific doses and their frequency are, to some extent, dictated by the institution's ability to support the patient through episodes of neutropenia. Although dose intensity may be important, under certain circumstances a higher dose intensity may be achieved with a lower-dose regimen given more frequently, as opposed to a high-dose regimen that may result in infection and consequent treatment delays.

## Prognostic Factors

The most important prognostic factor for patients with the ESFT is the presence or absence of clinically detectable metastatic disease at diagnosis. [176,214] Although the majority of patients with these tumors likely has microscopic metastatic disease at diagnosis, the presence of tumor demonstrable by clinical imaging techniques decreases the likelihood of long-term survival, from between 50% and 70% in modern series to less than 30%. [185] This stark dichotomy exists for most of the other childhood sarcomas and suggests that metastatic tumors are not just those detected later but are, in fact, biologically different from those still localized at diagnosis. The prognosis in patients whose disease is disseminated appears to depend additionally on the metastatic pattern of the disease. [299] The European intergroup found in patients with primary lung metastases that the 5-year and 10-year relapse-free survival rates were 36% and 30%, respectively. This finding contrasts to patients with metastatic disease to bone and bone marrow, for whom the 10-year EFS was 16% ($p$ = .0001).[300]

Among patients with localized tumors, primary site is related to outcome, with the least favorable site being the pelvis and most favorable sites distal bones, ribs, and cutaneous and subcutaneous sites.[163,164,176,208,301] Tumor size also has been found to be an important prognostic factor in some studies, although this criterion still is fairly controversial. The CESS-81 study demonstrated that patients with localized Ewing's sarcoma of bone smaller than 100 cc had DFS significantly better than that in patients with tumors larger than 100 cc.[181] Analysis of prognostic factors in CESS-86 confirmed large tumor volume to be the one factor influencing prognosis most significantly, but the tumor volume found to be critical and of poor significance was a volume greater than 200 cc. [164] Bacci et al.[163] did not find tumor volume, at least at 100 mL, to correlate with prognosis. Age at diagnosis was a significant prognostic factor in IESS-1 and IESS-2, with younger patients faring better than older patients in both studies.[185,208,273] Craft et al.[302] reported that patients who had the ESFT and were younger than 10 years had a prognosis better than that of older patients (relapse-free survival of 86% for younger patients vs. 55% for older patients). [302] This finding has been confirmed in other recent studies. [163,303]

Patients with elevated serum lactate dehydrogenase levels fare worse than do those with normal levels. [163,189,214] As discussed, the degree of neural differentiation no longer appears to be of prognostic significance. Rather, histologic response to chemotherapy after surgical resection appears to be an important prognostic indicator.[162] Additionally, as detailed, patients with tumors expressing a type 1 EWS-FLI1 transcript appear to have a better outcome. [89,93]

## Treatment of Recurrent Disease

Patients with a suspected recurrence must be evaluated fully both at the primary site and for the presence of metastatic disease before a plan of treatment can be formulated. The majority of patients with local treatment failure have concomitant gross or microscopic metastatic disease. The detection and treatment of a local recurrence may be difficult. Imaging studies often are not helpful in determining local recurrence, because interpreting clinical and radiographic examinations of irradiated areas is difficult. MRI has not proven as useful as originally hoped because of the marked skin and muscle changes after irradiation or surgery. [304] A persistent soft tissue mass often may be a residual fibrotic mass rather than a tumor. Determination of intraosseous recurrence is even more difficult. Plain radiographs show a variety of responses and patterns of reossification. If cortical destruction or increasing lysis occurs, an intraosseous recurrence must be suspected. Bone scans that show markedly increased activity suggest local recurrence. Second-look bone biopsies are associated with a high rate of local morbidity, wound problems, and secondary fracture and are fraught with the problem of sampling error.

The prognosis for patients with recurrent disease is poor. The length of survival depends on the site and extent of recurrence, the aggressiveness of the tumor, the previous treatment, and time to failure. The likelihood of response to retreatment increases with increasing duration of disease control. If disease progression or recurrence occurs during the initial therapy, the likelihood of a subsequent response to chemotherapy is nil. [284]

The choice of chemotherapeutic agents is dictated in part by affected patients' prior treatment. If such patients have not yet received agents with known activity against the ESFT, these agents should be used first. For example, if a patient previously has received VDC plus A alone, ifosfamide and etoposide should be considered at the time of recurrence, because they have demonstrated activity in this situation. [269] For those patients who have received intensive treatment with all known active agents that fails on or near the completion of therapy, retreatment with previously used agents is likely to be of little more than transient benefit. Such patients should be considered for entry into phase I or phase II new-drug studies. [305] The St. Jude group treated the only series of patients reported in the literature to have achieved significant benefit from retreatment with previously used agents. [284] This group reported that 0 of 18 patients with relapsing disease on therapy achieved a second complete response, versus 26 of 34 patients whose tumors recurred while they were off treatment, with 12 remaining in second remission from 5 months to 19 years from relapse. In some of the patients, third and even fourth remissions were obtained. The majority of the patients with favorable outcomes were those with relapse of a single pulmonary nodule. In many of them, the second complete response was obtained with surgery. Little information is available regarding the utility of resection of pulmonary nodules in patients with recurrent Ewing's sarcoma. Its value has been documented in such tumors as osteosarcoma but is less clear in cases of the ESFT. Bacci et al.[306] reported that 5 of 12 patients survived 3 to 14 years after pulmonary surgery for lung relapse of Ewing's sarcoma.

Management of a local recurrence may include surgery or radiotherapy (or both). A local recurrence in the extremity may require amputation, because surgery in a heavily irradiated field has little chance of success, especially if a prosthesis is required. If the recurrence involves the pelvis or proximal femur, a hemipelvectomy often is required. Local recurrence outside of the original irradiation field (marginal recurrence) may be salvaged with additional irradiation [245] or surgery. Sparse experience is reported for irradiation salvage of local recurrences after surgery to the primary lesion. The suitability of such cases for salvage by irradiation depends on limb function at the time of recurrence, the presence of a pathologic fracture, the ability to spare a strip of irradiated tissue, the ability to define accurately the recurrence with imaging studies, the exact anatomic location of the recurrence, and the age of an affected patient. Such decisions are best individualized and made after discussions among the pediatric oncologist, radiation oncologist, orthopedic surgeon, rehabilitation physician, patient, and family.

The European Bone Marrow Transplant Solid Tumor Registry analyzed information regarding 31 patients with relapsed ESFT. [286] These patients at first relapse were treated with megatherapy followed by bone marrow or peripheral stem-cell rescue between 1982 and 1992. Patients in second complete remission achieved a 32% EFS rate at 5 years. Local relapse was associated with a prognosis worse than that of distant recurrence and, not surprisingly, a favorable outcome was limited to relapsed patients with localized disease at diagnosis. Time to relapse in this study was not recognized as a prognostic factor. Others have attempted to use high-dose therapy with autologous stem-cell rescue for relapsed Ewing's sarcoma (Table 33-8). At this time, the data are too heterogenous to interpret.

## Complications of Treatment

The acute and chronic effects of the chemotherapeutic agents used to treat the ESFT are described in Chapter 10 and Chapter 9. Of particular concern for patients with primary tumors of the chest wall are the combined effects of doxorubicin, cyclophosphamide, and radiation to the heart, which produce a higher incidence of myocardial damage and congestive heart failure. The incidence of this complication may be decreased by the use of continuous-infusion doxorubicin [307] or the cardioprotector ICRF-187 (dexrazoxane, Zinecard).[308] A statistically significant increase in the risk of toxicity is associated with the combination of high individual dose and shorter interval between doxorubicin doses as measured by the dose intensity. [309] All patients who have received doxorubicin should have routine surveillance of

their cardiac function, because late congestive heart failure is increasingly being observed.

Toxicities of high-dose irradiation given in conjunction with intensive chemotherapy can be separated into acute and late complications. The severity of both can be limited by careful technique. Acute reactions are those occurring during or shortly after completion of radiotherapy. The most prominent acute side effects occur in tissues in the radiation field with rapidly dividing cells. Therefore, desquamation of the skin, mucositis, diarrhea, proctitis, or dysuria may be seen. The severity of these effects depends on the amount of normal tissue in the radiation field, radiation fraction size, and the timing of chemotherapy in relation to the radiation. Actinomycin-D and doxorubicin enhance acute radiation reactions. If these agents are to be administered during a course of radiotherapy, a planned break of several days in the radiation treatments may help to limit the severity of the interaction. Acute reactions usually are self-limited and resolve within 10 to 14 days of completion of irradiation. Dry desquamation can be managed with skin cream to soothe and moisten the affected area. When this reaction appears, a short break in the radiotherapeutic schedule may prevent progression to moist desquamation; if the latter occurs, a radiation treatment break and clean, wet soaks to the affected area three times daily are necessary. Mucositis and diarrhea require appropriate hydration and supportive care.

Late reactions are those occurring months to years after completion of a course of irradiation. The pathophysiology of late effects is understood incompletely but may be related to radiation injury to less rapidly proliferating supportive stroma or normal tissue. [310] Their severity is not always predicted by the severity of acute effects.

Reports of late functional deficits related to the combination of irradiation and intensive chemotherapy vary substantially in the frequency and severity of limitations reported.[243,252,311,312,313] and [314] Technique, treatment volume, and total dose are related to functional outcome, as are patient age, pretreatment functional status, presence of pathologic fracture at presentation, and the prescription and adherence to a rehabilitation program. Younger, prepubertal children are at greater risk of suffering radiation-induced arrest of bone growth. [313] Doses of 60 Gy or greater are associated with more complications, including markedly increased induration and fibrosis.[311,312,313,314] and [315] Circumferential irradiation of the extremity has been associated uniformly with fibrosis, edema, and poor function. [243,311] Late contractures are more apparent in less physically active patients. Higher radiation doses and a greater extent of cortical bone destruction by tumor or biopsy procedure have been associated with a higher frequency of fractures in the radiation field. [311,316] Weight-bearing lower extremities are at greater risk for complications than are the upper extremities.

An older series in which radiation dose, volume, and technique differed considerably from current standards described major radiation-related complications in 29% of patients. The most pronounced deficits occurred in the dose range of 6,500 to 7,800 roentgens. This finding contrasts to recent series that show that excellent functional results can be obtained in the majority of patients irradiated for Ewing's sarcoma. Jentzsch [311] reported that 13 of 22 patients treated with 50 Gy to the entire bone using shaped fields in conjunction with chemotherapy had essentially normal limb function with no more than minimal functional limitation or leg length discrepancy; only 1 patient had a functional impairment severe enough to warrant an amputation.

Sparing of uninvolved epiphyseal plates minimizes leg shortening secondary to irradiation. [315] The application of CT-directed treatment planning, by limiting the volume of normal tissue in the field, appears to offer further improvements in functional outcome. Appropriate case selection and application of careful radiotherapy technique in a series of patients with sarcomas of the hand and foot resulted in normal function in five of seven patients and only minor impairment in the remaining two. Experience with twice-daily radiotherapy (120 cGy twice daily to 50.4 to 60 Gy) for Ewing's sarcoma has demonstrated excellent functional outcome. [252] No pathologic fracture was seen in 18 patients with extremity primary lesions. Eight patients with extremity primary lesions underwent a formal evaluation of limb function between 2 and 7 years after radiotherapy. One patient showed a minor (1.5-cm) leg length discrepancy. Six of eight patients showed slight difference in extremity circumference, with an average circumference deficit of 0.87 cm in the treated extremity. Essentially, no detectable fibrosis or noticeable stiffness occurred in the treatment field.

An active physical therapy program should be established for each patient to maximize functional outcome. The program should include active joint extension and flexion. Rehabilitation should be started as soon as possible and should include weightbearing after involved bone has healed adequately (see Chapter 45). The patient should continue on a physical therapy regimen even after the completion of treatment to maximize long-term limb function. High-impact sports that pose the risk of traumatic fracture should be avoided during and after treatment.

The NCI completed a comprehensive case-control study of late effects in patients who had the ESFT and were treated at the NCI from 1965 to 1992. The study demonstrated multiple late effects in the ESFT survivors as compared to sibling controls. It also demonstrated that long-term survivors of the ESFT had educational achievements and income similar to those of their siblings; however, they were less likely than sibling controls to be employed full time. A lower likelihood of marriage was seen in this study, and fewer case subjects had children. Survivors of the ESFT reported multiple cancer- and treatment-related difficulties, such as hair and skin changes, lung problems, neurologic problems, visual difficulties, second malignancy, and amputation. Functional status, measured by Karnofsky performance scale, also was affected adversely in case subjects. Case subjects did not differ from sibling controls in health care insurance status or in use of health services.

Several studies have estimated an increased cumulative risk of secondary malignancies after Ewing's sarcoma. [19,217,301] Radiation-induced osteosarcoma and therapy-related (alkylating agents and epipodophyllotoxins) acute myeloblastic leukemia (AML) are the most frequent second malignant neoplasms reported. [317] In a recent multi-institutional review of 266 survivors of Ewing's sarcoma with a median follow-up duration of 9.5 years, the estimated cumulative incidence rates at 20 years for any second malignancy and for secondary sarcoma were 9.2% (standard deviation, 2.7%) and 6.5% (standard deviation, 2.4%), respectively. [217] The median latency to the diagnosis of the second malignancy was 7.6 years (range, 3.5 to 25.7). Sixteen patients developed second malignancies, and the majority (n = 10) developed sarcomas: five osteosarcomas, three fibrosarcomas, and two malignant fibrous histiocytomas. All the secondary sarcomas occurred at or near the primary tumor site of Ewing's sarcoma and within the primary irradiated field. The cumulative incidence rate of secondary sarcoma was radiation dose–dependent, with no secondary sarcomas developing among patients receiving less than 48 Gy. Six other malignancies were reported: AML, acute lymphoblastic leukemia, meningioma, bronchoalveolar carcinoma, basal cell carcinoma, and carcinoma *in situ* of the cervix. Recently, Dunst et al.[317] reported on the CESS-studies (CESS 81 and CESS 86) and second malignancies. From 1981 through 1991, 674 patients were registered in the two sequential multi-institutional Ewing's sarcoma trials. The median follow-up at the time of the analysis was 5.1 years; the maximum follow-up was 16.5 years. Eight of 674 patients developed a second malignancy. Four of these were AML: one myelodysplastic syndrome and three sarcomas. The interval between diagnosis of Ewing's sarcoma and the diagnosis of the second malignancy was 17 to 78 months for the four AMLs, 96 months for the myelodysplastic syndrome, and 82 to 136 months for the three sarcomas. The cumulative risk of a second malignancy was 2.9% after 10 years and 4.7% after 15 years. All three patients with secondary sarcomas had received radiotherapy. The authors noted the relatively low risk of second tumors in their irradiated patients, at least in the first 10 to 15 years after diagnosis, and this finding may have been derived from those patients who were treated with doses from 36 to 60 Gy.[317] Their data demonstrating a cumulative risk of fewer than 5% sarcomas over the first 10 to 15 years are similar to the result cited by Kuttesch et al.[217] for this dose range. These studies demonstrate that long-term oncologic follow-up is necessary in this group of patients.

## FUTURE CONSIDERATIONS

As discussed in detail, we now have a better understanding of the nature of the molecular mechanisms that cause the ESFT. Accumulated data indicate that the rearrangement of the *EWS* gene with either the *ets* oncogene *FLI1* or, in rarer cases, one of the other related *ets* transcription factor genes constitutes a rate-limiting step in Ewing's sarcoma pathogenesis. This knowledge allows speculation on possible specific treatment strategies that have as their common mechanism the prevention of the production or activity of the protein that results in the malignant process. For instance, production of the protein may be prevented through antisense blockade of the chimeric mRNA. The activity of the protein may be inhibited through specific monoclonal antibodies. Such monoclonal antibodies directed toward the chimeric protein also may be specific for ESFT cells, allowing the *ex vivo* purging of marrow or stem-cell collections or the *in vivo* targeting of tumors with radioisotopes. The chimeric protein may be the target also of a tumor vaccine. Another potential avenue of investigation is development of an assay that allows the screening of drugs to identify one that specifically interferes with the activity of this aberrant protein. We are at the beginning of a new era in the treatment of the ESFT. This disease may serve as a model for what can be accomplished through the molecular genetic-based therapy of cancer.

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# 34

# OTHER SOFT TISSUE SARCOMAS OF CHILDHOOD

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## INTRODUCTION

The nonrhabdomyosarcomatous soft tissue sarcomas (NRSTSs) are a rare and heterogeneous group of neoplasms of mesenchymal origin, which account for approximately 5% of all cancers in patients younger than 20 years.[1] The incidence of specific subtypes of soft tissue sarcomas is age dependent. For example, rhabdomyosarcoma accounts for 60% of cases of soft tissue sarcomas in children younger than 5 years; in contrast, more than three-fourths of all soft tissue sarcomas in patients aged 15 to 19 years are NRSTSs (Fig. 34-1, Fig. 34-2, and Fig. 34-3).[1] Furthermore, the distribution of histologic subtypes of NRSTS is also age

dependent (Fig. 34-2 and Fig. 34-3). Fibrosarcomas predominate in children younger than 1 year, whereas synovial sarcomas and malignant peripheral nerve sheath tumors (MPNSTs) are more frequently encountered in patients older than 10 years (Table 34-1).[1,2]

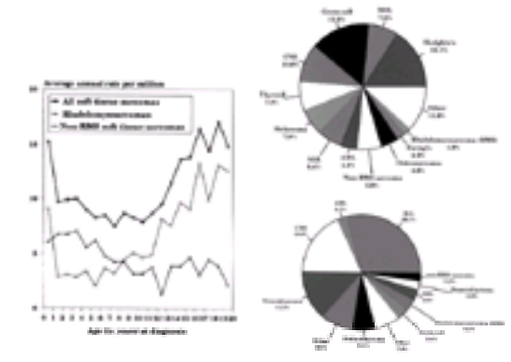

**FIGURE 34-1.** Graph **(A)** shows the incidence rates for soft tissue sarcomas by single year of age. Note the rising incidence of nonrhabdomyosarcomatous (non-RMS) soft tissue sarcomas among children older than 10 years. **(B)** shows the incidence of specific cancers for the period 1986–1995 in children aged 15 to 19 years. Note that non-RMS soft tissue sarcomas account for nearly 6% of all cancers in this age group. In contrast, rhabdomyosarcoma is the predominant soft tissue tumor in children younger than 5 years **(C)**. ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CNS, central nervous system; NHL, non-Hodgkin's lymphoma.

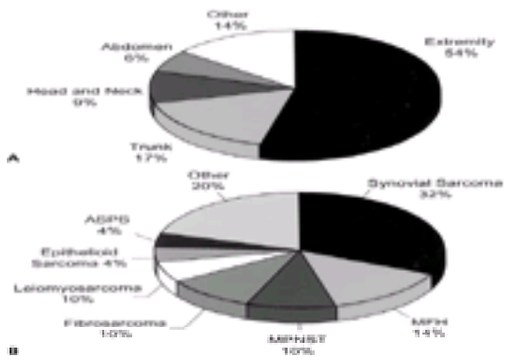


**FIGURE 34-2.** Distribution by histologic subtype **(B)** and primary tumor site **(A)** in children with surgically resected (groups I and II) nonrhabdomyosarcomatous soft tissue sarcoma. ASPS, alveolar soft part sarcoma; MFH, malignant fibrous histiocytoma; MPNST, malignant peripheral nerve sheath tumor. (From Spunt SL, Poquette CA, Hurt YS, et al. Prognostic factors for children and adolescents with surgically resected nonrhabdomyosarcoma soft tissue sarcoma: an analysis of 121 patients treated at St. Jude Children's Research Hospital. J Clin Oncol 1999;17:3697–3705; and Pratt CB, Pappo AS, Gieser P, et al. Role of adjuvant chemotherapy in the treatment of surgically resected pediatric nonrhabdomyosarcomatous soft tissue sarcomas: a Pediatric Oncology Group study. J Clin Oncol 1999;17:1219, with permission.)

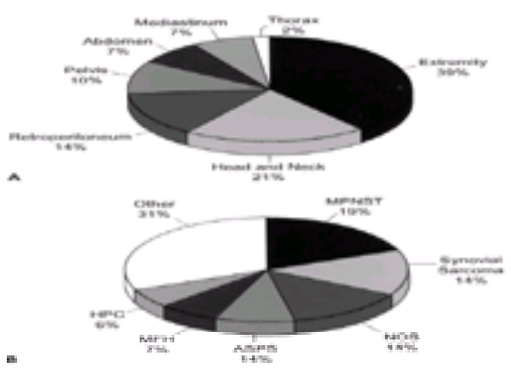


**FIGURE 34-3.** Distribution by histologic subtype **(B)** and primary site **(A)** among children with unresected (group III) or metastatic (group IV) nonrhabdomyosarcomatous soft tissue sarcomas. ASPS, alveolar soft part sarcoma; HPC, hemangiopericytoma; MFH, malignant fibrous histiocytoma; MPNST, malignant peripheral nerve sheath tumor; NOS, not otherwise specified. [From Pappo AS, Rao BN, Jenkins JJ, et al. Metastatic nonrhabdomyosarcomatous soft-tissue sarcomas in children and adolescents: the St. Jude Children's Research Hospital experience. Med Pediatr Oncol 1999;33:76–82; Spunt SL, Hill DA, Motosue AM, et al. Clinical features and outcome of children with unresected non-rhabdomyosarcoma soft tissue sarcoma (NRSTS). Med Ped Oncol 2000;35:279(abst); and Lack EE. Leiomyosarcoma in children: a clinical and pathologic study of 10 cases. Pediatr Pathol 1986;6:181, with permission.]

**TABLE 34-1. CLINICAL FEATURES OF THE COMMON SOFT TISSUE SARCOMAS IN CHILDREN**

Because NRSTSs are more common in adults, with approximately 6,000 new cases per year, much of the information regarding their natural history and treatment has been derived from single-institution or cooperative adult trials. In some specific circumstances, however, the prognosis for children with individual soft tissue sarcomas is much better than that for adults, resulting in markedly different treatment recommendations.[3,4,5,6] and [7] The difference in prognosis is most pronounced for infants and young children, whose tumors often have a benign behavior and excellent prognosis with surgery alone. In contrast, NRSTS that occur in adolescents often behave more like the sarcomas that occur in adult patients,[5,6,7,8] and [9] and their management reflects that used in adults.

## EPIDEMIOLOGY, GENETICS, AND BIOLOGY

Specific chromosome translocations define many of the soft tissue sarcomas in children (Table 34-2).[10,11] and [12] For example, the t(X;18)(p11; q11) occurs in more than

90% of synovial sarcomas.[12,13] Molecular studies reveal that two novel genes are rearranged: SYT on 18q11 and SSX on Xp11. [14] Furthermore, two alternative forms of the SYT-SSX fusion transcript, SYT-SSX1 and SYT-SSX2, have been described and are related to histologic and clinical features of the tumor: SSX1 associated with both biphasic and monophasic tumors and SSX2 with monophasic tumors.[15,16] This suggests that the two altered genes have different quantitative or qualitative effects on the epithelial differentiation of this tumor. [16] Liposarcoma is similarly characterized by a translocation t(12;16)(q13; p11), resulting in the fusion of a transcription factor essential for adipocytic differentiation to EWS or TLS, known oncogenes. [16,17] and [18] EWS is similarly involved in fusion genes associated with other sarcomas, including Ewing's sarcoma and desmoplastic small round cell tumor.[12,20,21]

| Tumor | Translocation | Fusion protein |
|---|---|---|
| Clear cell sarcoma | t(12;22)(q13;q12) | EWS-ATF-1 |
| Extraskeletal myxoid chondrosarcoma | t(9;22)(q22;q12) | EWS-CHN |
| | t(9;17)(q22;q11.2) | RBP56-CHN |
| Synovial sarcoma | t(X;18)(p11;q11) | SYT-SSX1, SSX2 or SSX4 |
| Myxoid liposarcoma | t(12;16)(q13;p11) | TLS-CHOP |
| | t(12;22)(q13;q12) | EWS-CHOP |
| | t(12;22;16)(q13;q12;p11) | EWS-CHOP |
| Congenital fibrosarcoma | t(12;15)(p13;q25) | ETV6-NTRK3 |
| Dermatofibrosarcoma protuberans | t(17;22)(q22;q13) | COL1A1-PDGF |
| Malignant peripheral nerve sheath tumor | Loss or rearrangement of 10p, 11q, 17q, and 22q | ? |
| Inflammatory myofibroblastic tumor | 2p rearrangements | TPM3-ALK |
| | | TPM4-ALK |
| Alveolar soft part sarcoma | t(X;17)(p11.2;q25) | ASPL-TFEB |
| Leiomyosarcoma | 13q rearrangements, loss of 1q, 4q, 11p, and 22q | ? |

From Ladanyi M, Lui MY, Antonescu CR, et al. Cloning of the der(17)t(X;17)(p11.2;q25) of alveolar soft part sarcoma identifies the ASPL-TFE3 gene fusion, a new molecular diagnostic marker. Mod Pathol 2001;14(abstr 30); Fletcher CDM, Dal Cin P, de Wever I, et al. Correlation between clinicopathological features and karyotype in spindle cell carcinoma: a report of 130 cases from the CHAMP study group. Am J Pathol 1998;154:1841–1847; Shapiro DN, Chen CH. Cytogenetics and the biologic basis of sarcomas. Curr Opin Oncol 2000;12:310–322; and Lawrence B, Perez-Atayde A, Hibbard MK, et al. TPM3-ALK and TPM4-ALK oncogenes in inflammatory myofibroblastic tumors. Am J Pathol 2000;157:377–384, with permission.

**TABLE 34-2. CHROMOSOMAL TRANSLOCATIONS AND CURRENTLY IDENTIFIED FUSION PROTEINS IN SOFT TISSUE TUMORS**

Many NRSTSs are associated with loss of heterozygosity, the clearest clinical example being the association of these tumors with the familial cancer syndrome described by Li and Fraumeni.[22,23,24] and [25] Further supporting this hypothesis is the strong association of MPNSTs with von Recklinghausen's disease (neurofibromatosis type I, or NF1), a common autosomal dominant disorder associated with abnormalities mapped to 17q11.2. [26,27] Furthermore, nerve sheath tumor has been shown by direct sequence analysis to have a point mutation at the p53 locus at 17p13, suggesting a role in pathogenesis. [27] Homozygous gene deletions of both the long and short arms of chromosome 17 have been observed in MPNSTs arising in patients with neurofibromatosis. [27] A somatic deletion of the NF1 gene has also been reported in an MPNST not associated with NF1. [28] Because other sarcomas, including rhabdomyosarcoma and malignant fibrous histiocytoma (MFH), have also been associated with neurofibromatosis, it is very likely that loss of heterozygosity of a tumor suppressor gene on chromosome 17 or at other sites is at least a factor in the development of most of the sarcomas in this genetically inherited autosomal dominant syndrome. Furthermore, it is likely that these sarcomas, especially MPNSTs, arising in individuals who do not have neurofibromatosis also have loss of heterozygosity of a tumor suppressor gene or genes within the tumor cells, caused by two somatic mutations. Further support for this hypothesis comes from the observation that the MPNSTs arising in patients with neurofibromatosis occur at an earlier age than those arising in patients without neurofibromatosis. [29,30,31,32] and [33]

NRSTSs, most commonly MFH, may be associated with prior irradiation for a primary tumor.[34] The origin of these tumors may be mediated in part through genetic events induced by the radiation. The observation that radiation is implicated in the development of sarcomas in patients with the Li-Fraumeni family cancer association and hereditary retinoblastoma further supports this hypothesis. Leiomyosarcomas arise both within and outside the radiation fields of children with hereditary retinoblastoma, however, suggesting that multiple factors are involved in the etiology of this tumor. Furthermore, leiomyosarcoma has been observed in children infected with the human immunodeficiency virus (HIV) and the Epstein-Barr virus (EBV), [36,37] in a patient with leukemia,[38] and in a child undergoing immunosuppression to prevent renal allograft rejection.[39] This raises the possibility that the occurrence of this soft tissue sarcoma may be related to a lack of immunocompetence of the host or to a retroviral infection.

There are dramatic differences in biology and natural history of soft tissue sarcomas when they arise in infants and young children compared to those that arise in adults. For example, infantile fibrosarcoma, in contrast to its adult counterpart, rarely metastasizes and is almost always cured if complete surgical removal can be accomplished.[40,41,42] and [43] The consistent genetic events found with infantile fibrosarcoma, but not with the fibrosarcoma arising in adults, are an important clue that there are fundamental differences between these two clinical presentations of fibrosarcoma. [44] Although infantile hemangiopericytoma rarely metastasizes and usually can be successfully managed by surgery alone,[45] the adult form of hemangiopericytoma is highly malignant in behavior and frequently metastasizes. [46,47] These observations strongly suggest that in spite of a very similar histologic appearance, there are important biologic differences between tumors arising in infants and those arising in adults. The prognosis and natural history of soft tissue sarcomas arising in older children and adults are very similar, suggesting a common biology.

## CLINICAL PRESENTATION

NRSTSs may arise in any part of the body; however, the most common sites are the extremities and trunk (Fig. 34-2). Most soft tissue sarcomas present as painless, asymptomatic masses; however, if symptoms occur, they are usually due to local invasion of adjacent structures. Invasion of cranial and peripheral nerves often results in pain or weakness in the distribution of the nerve. MPNSTs typically arise in a peripheral nerve, often the sciatic nerve, with both motor and sensory involvement. Systemic symptoms such as fever, night sweats, and weight loss are rare, although occasionally they are seen in patients with widely metastatic disease. Hypoglycemia has been seen occasionally with advanced hemangiopericytoma and other soft tissue sarcomas; the insulin-like growth factor-1 receptor has been implicated in the pathogenesis of this complication.[48] Hyperglycemia has been observed in association with fibrosarcoma of the lung. [49] Hypophosphatemic rickets has been associated with hemangiopericytoma.[50]

## STAGING

There are a variety of staging systems for NRSTS. Although the staging systems used for adults differ from those used in pediatric patients, they all allow identification of subgroups of patients who are at different risks of treatment failure. For adult NRSTS, the most recent American Joint Committee on Cancer unified staging system[51] and the Memorial Sloan-Kettering Cancer Center staging system incorporate histologic grade, tumor size, compartmental localization, and metastatic disease to define the risk of relapse. The Surgical Staging System of the Musculoskeletal Tumor Society is based on tumor grade and tumor compartment status only. Recently, these staging systems were evaluated in 300 adults with newly diagnosed nonmetastatic soft tissue sarcoma of the lower extremity. This report concluded that grade, depth, and size were the most important predictors of clinical outcome.[52] The Memorial Sloan-Kettering Cancer Center staging system proved to be superior to the other two systems for predicting metastases-free survival. In this staging system, adults with no adverse prognostic factors as defined by histologic grade, depth, and size had an estimated 5-year metastases-free survival of 100%, whereas those with three adverse factors had a 49% 5-year metastases-free survival.[52]

Pediatric NRSTSs have traditionally been staged according to the Intergroup Rhabdomyosarcoma Study Group surgicopathologic staging system and the International Union Against Cancer staging systems (Table 34-3 and Table 34-4).[53,54] The impact of the extent of residual tumor after initial surgical procedures and the presence or absence of metastases is presented in Table 34-3.[53] According to the International Union Against Cancer staging system, which incorporates size (a or b), nodal status (N0 or N1), invasiveness (T1 or T2), and the presence or absence of metastases, patients with localized small noninvasive tumors (T1a) fare better than those with invasive large tumors (T2b) (Table 34-5).[55]

| Group | Description | 5-Yr event-free survival (%) | 5-Yr survival (%) |
|---|---|---|---|
| I | Localized disease, completely resected | 72–83 | 88–90 |
| II | Microscopic residual<br>Completely resected with nodes<br>Nodes involved with microscopic residual | 65–72 | 84–88 |
| III | Incomplete resection | —[a] | 35–54[a] |
| IV | Distant metastases | 15 (2-yr) | 34 (2-yr) |

[a]No data on N1 patients; however, the new American Joint Committee on Cancer staging system for adults considers N1 patients as stage 4 (5-yr survival 34%).
From Spunt SL, Poquette CA, Hurt YS, et al. Prognostic factors for children and adolescents with surgically resected nonrhabdomyosarcoma soft tissue sarcoma: an analysis of 121 patients treated at St. Jude Children's Research Hospital. J Clin Oncol 1999;17:3697–3705; Pratt CB, Pappo AS, Gieser P, et al. Role of adjuvant chemotherapy in the treatment of surgically resected pediatric nonrhabdomyosarcomatous soft tissue sarcomas: a Pediatric Oncology Group study. J Clin Oncol 1999;17:1219; and Pratt C, Maurer H, Gieser P, et al. Treatment of unresectable or metastatic pediatric soft tissue sarcomas with surgery, irradiation, and chemotherapy: a Pediatric Oncology Group study. Med Pediat Oncol 1998;30:201–209, with permission.

**TABLE 34-3. INTERGROUP RHABDOMYOSARCOMA STUDY GROUP SURGICOPATHOLOGIC STAGING SYSTEM AND CLINICAL OUTCOME ACCORDING TO CLINICAL GROUP**

| Stage | Tumor, node | Metastasis |
|---|---|---|
| 1 | T1a-T1b, N0 | M0 |
| 2 | T2a-T2b, N0 | M0 |
| 3 | Any T, N1 | M0 |
| 4 | Any T, Any N | M1 |

a, tumor ≤5 cm in greatest dimension; b, tumor >5 cm in greatest dimension; M1, distant metastases; N0, no regional nodal metastases; N1, regional nodal metastases; T1, tumor limited to organ or tissue of origin; T2, tumor invades contiguous organs or tissues and/or with adjacent malignant effusion.
From Lawrence W Jr, Gehan EA, Hays DM, et al. Prognostic significance of staging factors of the UICC staging system in childhood rhabdomyosarcomas: a report from the Intergroup Rhabdomyosarcoma study (IRS-II). J Clin Oncol 1987;5:46, with permission.

**TABLE 34-4. CLINICAL INTERNATIONAL UNION AGAINST CANCER STAGING SYSTEM FOR PEDIATRIC SOFT TISSUE TUMORS**

| | 5-Yr survival (%) | 5-Yr progression-free survival (%) |
|---|---|---|
| T1 | 96 | 87 |
| T2 | 70 | 51 |
| a | 97 | 52 |
| b | 77 | 55 |

a, tumor ≤5 cm in greatest dimension; b, tumor >5 cm in greatest dimension; T1, tumor limited to organ or tissue of origin; T2, tumor invades contiguous organs or tissues and or with adjacent malignant effusion.
From Spunt SL, Poquette CA, Hurt YS, et al. Prognostic factors for children and adolescents with surgically resected nonrhabdomyosarcoma soft tissue sarcoma: an analysis of 121 patients treated at St. Jude Children's Research Hospital. J Clin Oncol 1999;17:3697–3705, with permission.

**TABLE 34-5. OUTCOME FOR LOCALIZED PEDIATRIC NONRHABDOMYOSARCOMATOUS SOFT TISSUE SARCOMAS ACCORDING TO THE INTERNATIONAL UNION AGAINST CANCER STAGING SYSTEM**

Histologic grading has been used in both adult and pediatric studies as an adjunct to clinical staging because it is highly predictive of clinical outcome. [55],[56] and [57] The system developed by the National Cancer Institute of the United States (NCI) by Costa and colleagues stratifies soft tissue sarcoma into three different grades based on histologic subtype and a composite of histopathologic parameters that includes tumor necrosis, cellularity, pleomorphism, and mitosis. [55]

The Pediatric Oncology Group (POG) developed and prospectively tested a pediatric grading system for NRSTS [58] (Table 34-6 and Table 34-7) based on the histopathologic system developed by Costa and colleagues.[55] This grading system identified three different grades of tumors based on histopathologic subtype: amount of necrosis, number of mitoses, and cellular pleomorphism (Table 34-6). Infantile tumors such as hemangiopericytoma and fibrosarcoma are considered grade 1 in this classification despite their aggressive appearance on histologic examination. This is the system most commonly used to grade pediatric NRSTSs.

| Grade | Description |
|---|---|
| 1 | Myxoid and well-differentiated liposarcoma<br>Well-differentiated or infantile (age ≤4 years) fibrosarcoma<br>Well-differentiated or infantile (age ≤4 years) hemangiopericytoma<br>Well-differentiated malignant peripheral nerve sheath tumor<br>Angiomatoid malignant fibrous histiocytoma<br>Deep seated dermatofibrosarcoma protuberans<br>Myxoid chondrosarcoma |
| 2 | Soft tissue sarcomas in which<br>•<15% of the surface area shows necrosis<br>•The mitotic count is <5/10 high power fields using a 40× objective<br>•Nuclear atypia is not marked<br>•The tumor is not markedly cellular* |
| 3 | Tumors with the following diagnosis<br>Pleomorphic or round cell liposarcoma<br>Mesenchymal chondrosarcoma<br>Extraskeletal osteogenic sarcoma<br>Malignant triton tumor<br>Alveolar soft part sarcoma<br>Any other sarcoma not in grade 1 with ≥15% necrosis or ≥5 mitoses/10 high power field using a 40× objective |

*Necrosis and mitotic count are by far the most important parameters in making this assessment. The other parameters are of borderline significance and may be helpful in a case that is difficult to grade using necrosis and mitotic count alone. Specific diagnoses included in grades 1 or 3 are excluded from grade 2.
From Parham DM, Webber BL, Jenkins JJ III, et al. Nonrhabdomyosarcomatous soft tissue sarcomas of childhood: formulation of a simplified system for grading. Modern Pathol 1995;8:705–710, with permission.

**TABLE 34-6. PEDIATRIC ONCOLOGY GROUP HISTOLOGIC GRADING SYSTEM**

| Grade | 5-Yr survival (%) | 5-Yr event-free survival (%) |
|---|---|---|
| 1, 2 | 99 | 84–93 |
| 3 | 73 | 52–67 |

From Spunt SL, Poquette CA, Hurt YS, et al. Prognostic factors for children and adolescents with surgically resected nonrhabdomyosarcoma soft tissue sarcoma: an analysis of 121 patients treated at St. Jude Children's Research Hospital. J Clin Oncol 1999;17:3697–3705; and Pratt CB, Pappo AS, Gieser P, et al. Role of adjuvant chemotherapy in the treatment of surgically resected pediatric nonrhabdomyosarcomatous soft tissue sarcomas: a Pediatric Oncology Group study. J Clin Oncol 1999;17:1219, with permission.

**TABLE 34-7. CLINICAL OUTCOME FOR LOCALIZED PEDIATRIC NONRHABDOMYOSARCOMATOUS SOFT TISSUE SARCOMAS USING THE HISTOPATHOLOGIC GRADING SYSTEM PROPOSED BY THE PEDIATRIC ONCOLOGY GROUP**

## PROGNOSTIC FACTORS

Although the clinical outcome for children with completely excised NRSTS is excellent ( Table 34-8), more than 20% of these children eventually develop disease recurrence and ultimately die from their disease.[59,60 and 61] Identification of risk factors associated with an increased likelihood of tumor recurrence in patients with complete surgical excision disease is pivotal when selecting patients for adjuvant trials that incorporate chemotherapy, radiotherapy, or both ( Table 34-9). Adult trials have demonstrated that the risk factors for local recurrence differ from those for metastatic recurrence and death due to disease. The most significant adverse prognostic factor for distant recurrence and death is high histologic grade; however, the relative contribution of this variable to disease recurrence decreases over the first 30 months after initial surgery. The size of the lesion becomes as important as grade in determining the risk for distant recurrence thereafter. The number of adverse prognostic factors is also significantly associated with the risk of disease recurrence and death.

**TABLE 34-8. PEDIATRIC SERIES OF NONRHABDOMYOSARCOMA SOFT TISSUE SARCOMAS**

**TABLE 34-9. PEDIATRIC AND ADULT SERIES THAT HAVE ANALYZED PROGNOSTIC FACTORS IN NONRHABDOMYOSARCOMATOUS SOFT TISSUE SARCOMAS**

A relatively small number of pediatric trials, most of them retrospective in nature, have investigated the relative contribution of various prognostic factors in predicting clinical outcome.[59,62,63] In the largest retrospective single-institution study of its kind, investigators at St. Jude found that the risk factors associated with a local recurrence differed from those associated with distant relapse or survival. [59] In this series, clinical group, lack of radiotherapy use, large tumor size, and intra-abdominal primary site predicted local recurrence, whereas tumor size, invasiveness, and high histologic grade predicted distant failure. Other single-institution and cooperative group trials have also shown that histologic grade is an important predictor of distant recurrence. These findings are of significant relevance when planning prospective randomized multi-institutional trials. Patients at increased risk for local recurrence might benefit from novel approaches to local control, whereas those at increased risk for distant failure might benefit from adjuvant therapies including chemotherapy or anti-angiogenic agents. The risk factors associated with local and distant recurrence and those associated with survival in children with surgically resected NRSTS are summarized below. [59]

Factors associated with increased risk of local recurrence:

- Microscopically positive margins
- Intra-abdominal primary tumors
- No radiotherapy
- Tumor size >5 cm

Factors associated with increased risk of distant recurrence:

- Tumor size >5 cm
- Invasive tumors
- High histologic grade

Factors associated with decreased survival:

- Microscopic positive margins
- Tumor size >5 cm
- High histologic grade
- Intra-abdominal primary tumor site

Metastatic disease at the time of initial presentation occurs in approximately 15% of children with NRSTS. [62] The lung is the most common site of distant metastases, although metastases to bone, liver, and mesentery have also been reported. Lymphatic spread is rare with most histologies; however, it is commonly associated with high-grade lesions, synovial sarcoma, angiosarcoma, and epithelioid sarcoma histologies. The prognosis for patients with lymphatic metastases is comparable to that of patients with metastatic disease at other sites.

## GENERAL TREATMENT CONSIDERATIONS

The standard approach to management of these tumors in adults has undergone significant debate. The optimal management and treatment strategies are related to anatomic site and extent of both local and systemic disease. The relative rarity of these tumors and the heterogeneity of the histologic diagnoses entered onto these adult trials obfuscate to some degree the therapeutic results of these trials for specific tumor types. Because even smaller numbers of children develop NRSTS, it is essential that uniform treatment strategies be evaluated in multi-institutional trials.

The mainstay of therapy, as in adults, is surgical resection with or without radiotherapy. The primary tumor is excised with wide margins, and radiation therapy (RT) is usually added to the regimen if limb-sparing procedures are performed or the margins are close. [64,65 and 66] In some centers, the initial treatment is preoperative irradiation followed by surgery and then completion of RT using external beam and interstitial irradiation. [66,67] In other centers, preoperative intravenous chemotherapy

using a variety of regimens, or intraarterial chemotherapy consisting of cisplatin with or without doxorubicin (Adriamycin), is used to reduce the size of the local tumor and provide systemic therapy. This neoadjuvant chemotherapy is then followed by surgery; irradiation is used only if the surgical margins are inadequate. Although all of these approaches have resulted in excellent local control (less than 10% local failure rate), none has had a major impact on overall survival, [66,67,68 and 69] especially for large tumors.

Although the general approach to children with these tumors is often similar to that for adults, important differences exist. [6,7,8 and 9] First, the biology of the childhood NRSTS may differ significantly from that of the adult counterpart. Second, the morbidity of RT in a young and rapidly growing child may be much greater than that in an adult, depending on the site that requires irradiation. Third, successful limb-sparing procedures in young, growing children are more difficult to perform; however, newer techniques and expandable prostheses may allow a greater number of limbs of children to be salvaged. Finally, the long-term consequences of irradiation, especially if given at high dose, [70] and of chemotherapy are of greater concern in children whose potential survival after successful therapy is much longer. To achieve the goal of maximum tumor control with minimum morbidity in both the short and the long term, children with these tumors must be studied prospectively.

The most important theme of most reports is that the ability to surgically extirpate the tumor is the most critical prognostic factor. In a retrospective review of 121 surgically resected cases of childhood NRSTS from St. Jude Children's Research Hospital, the estimated 5-year survival was 89%, with 12.8% of patients experiencing a local failure and 11.8% a distant failure. [59] In contrast, only half of patients with unresected disease and 34% of those with metastatic disease were alive at 5 and 2 years, respectively. [62,63] Another study conducted by the POG revealed similar results with an 84% 5-year survival rate for children with surgically resected disease and a 4-year survival rate of 30% for patients with unresected or metastatic disease. [71]

## SURGERY

Much of the current approach regarding surgical management of these tumors has derived from the adult experience. [72,73,74 and 75] Surgery remains the mainstay of therapy for NRSTS. Surgical approaches vary by age, histology, site, and grade of the tumor. For example, the clinical behavior of infantile fibrosarcoma, infantile hemangiopericytoma, and angiomatoid MFH is different from that of other high-grade malignancies and therefore can often be treated without the use of mutilative surgery.

With adult NRSTS, local recurrence rates of less than 10% can be achieved with the use of surgery with or without irradiation. [67,76,77] In addition, most tumors that arise in the extremities can be managed successfully without amputation.[66] However, systemic metastases occur in up to 40% of adult patients who initially present with localized disease. The integration of effective systemic therapy into the management of children with NRSTS without compromising the local control that can be achieved with irradiation and surgery alone is essential if the overall outcome of these children is to be improved.

### Biopsy

A carefully planned diagnostic biopsy is very important to avoid misdiagnosis, delayed diagnosis, or complications that interfere with or in some cases even prevent limb salvage surgery. If an NRSTS is suspected, it is advisable to perform a thorough radiographic evaluation, including magnetic resonance imaging (MRI) of the primary site, before the biopsy is performed. In most instances a minimum of a fine-needle aspiration or Tru-Cut needle biopsy is warranted. A frozen section analysis of the specimen is desirable to be certain viable tumor rather than the necrotic portion of the tumor is obtained. Tru-Cut biopsies using diagnostic imaging for guidance are useful for intracavitary lesions. Although needle track recurrences have been documented, these are extremely rare in sarcomas. [78] Tru-Cut biopsies are also indicated for bulky, deep-seated lesions on the extremity, invasive truncal lesions, for those who present with metastatic disease, or where technical considerations do not favor an open incisional biopsy.

In most instances, an open biopsy is performed. Excisional biopsy is reserved generally for small (less than 2.5 cm) superficial lesions. In all other instances, an incisional biopsy is the approach of choice. In extremity lesions the incision is placed longitudinally or parallel to the neurovascular bundle. Transverse or inappropriately placed incisions can traverse multiple tissue compartments and may preclude limb salvage procedures. Similarly, an inappropriately placed incision on the trunk or head and neck may preclude closure. At the time of incisional biopsy, flaps should not be considered because they can contaminate multiple compartments. Meticulous hemostasis is mandatory and, if necessary, closed drains should be placed and brought out immediately adjacent to the most dependent area and in line within the incision. Should a hematoma develop, the entire area of the hematoma must be re-excised at the time of subsequent surgery. Most important, children with these lesions should only be evaluated, biopsied, and treated at institutions with appropriate surgical expertise, including limb-salvage techniques and pathologic support.

The exact margin required for local control remains to be determined. Based on the planes of dissection of the original sarcoma, there are four possible surgical margins.[75,79,80 and 81] An *intralesional margin* of resection is defined by the plane of dissection violating the pseudocapsule, and only a portion of the tumor is removed and obvious macroscopic tumor is left behind. Regrowth of the tumor is a certainty unless additional measures are taken. A *marginal margin* is defined by the pseudocapsule being the plane of dissection. When there are marginal margins, local recurrence rates range from 60% to 70%. A *wide resection* includes tumor, its pseudocapsule, and a margin of normal tissue removed in all directions *en bloc*. Quantitative measurements in centimeters are not implied in the definition of wide margins. Recurrence rates are 5% to 10% after this type of resection margin. *Radical margins* by definition are extracompartmental resection or resection of the whole soft tissue compartment. Recurrence rates are less than 5% when resections with radical margins are performed.

The amount of normal margin required to minimize the risk of local failure is unknown; however, a 1- to 2-cm margin after resection is usually preferred. For large, high-grade, and deep-seated tumors, the potential for local recurrence is high. The extent of normal tissue margin may become an important factor in preventing recurrence and the determining factor in deciding whether adjuvant therapy is delivered in these cases. Importantly, local recurrence is most often associated with inadequate treatment of the primary tumor with inadequate surgical margins.

Should diagnostic imaging studies, review of intraoperative sites, or pathologic examinations reveal that there is residual tumor or that the margins are close, it is recommended that the patient undergo reexcision of the primary site. In patients undergoing reexcision, residual disease is present in 30% to 40% of cases.

### Surgery for Extremity Tumors

Amputation is infrequently required in the current management of NRSTS of the extremity. [74,75,82,83,84 and 85] This is due to better surgical techniques and the low rate of recurrence when RT is used to treat marginal surgical margins. Further, amputation does not offer a clear survival benefit when compared to less extensive surgery followed by radiation. Nevertheless, amputation may be the only choice in a skeletally immature child or when there is obvious neurovascular involvement.

As previously mentioned, the primary goal of surgery is to achieve a wide local resection with adequate margins, which are considered to be approximately 2 cm. To ensure this 2-cm margin, careful palpation to gauge the extent of the tumor is done as the dissection proceeds. The previous biopsy site is excised with at least a 1-cm margin. Flaps are developed and complete resection obtained by either a wide or a compartmental resection. Although wide margins are obtained in most directions, in certain situations, especially around vessels and nerves, it may be possible to achieve a marginal margin of only a few millimeters. Subsequent RT is required in these cases for adequate local control. The adventitial sheath along the vessels or the perineural sheath adjacent to the tumor may be removed along with the specimen to obtain additional margin. If the vessel is grossly involved, resection and interposition of a prosthetic or venous graft may be considered. Here it is to be understood that the risk of infection poses the main threat to the graft. When tumor abuts bone, stripping the periosteum or marginal resection of the bone can be performed. The resultant defect can be bridged by allograft or vascularized fibula graft.

Sarcomas of the antecubital and the popliteal fossae present special problems. The complex neurovascular anatomy and loose connective tissue preclude wide resection of the tumor and close margins virtually always result. [82] Because of this, RT in the form of brachyradiotherapy (BRT), external beam RT (EBRT), or both are required for local control. Amputation for tumors in these sites is considered for treatment of local recurrence or when there is gross neurovascular involvement. In the skeletally immature child, successful local control with surgery, radiation, or both likely has a poorer functional outcome than when the tumor arises in a skeletally mature individual.[85]

When NRSTSs arise in the hand or foot, the close confines and tight compartments make resection marginal at best. Ray amputation of one or more digits may result in wider margins. RT is often necessary for optimal local control. Amputation of the foot may be required for large invasive tumors and local recurrences or when a poor functional outcome is anticipated. Amputation of the foot may also be necessary for adequate pain control. The recommended approach to tumors of the hand and foot is to achieve minimal surgical margins combined with subsequent RT. This approach maximizes local control and functional outcome without jeopardizing

overall survival.[85]

The incidence of nodal involvement in NRSTS is low.[86,87] In a collective review of more than 2,500 cases it was noted to be 3.9%. The incidence was dependent on the grade of the lesion, with an incidence of 0% for grade 1 lesions and only 2% for grade 2 tumors; however, the incidence rose to 12% for grade 3 tumors. Angiosarcoma and epithelioid sarcoma are the histologies most commonly associated with lymphatic metastases. Nodal metastasis of NRSTS in adults has been recognized to be associated with a poor outcome and is commonly treated with radical surgery. At St. Jude, 80 of 230 patients underwent lymph node dissection or biopsy of suspicious nodes defined as greater than 1.5 cm by imaging studies.[81,85] The nodes were positive in only ten patients, of whom eight had high-grade lesions. In light of these data, routine lymph node sampling is not recommended, but lymph nodes greater than 1.5 cm that are associated with large, high-grade primary lesions should be biopsied. Furthermore, any other large or suspicious node should also be reasonably considered for biopsy.

After conservative surgery in combination with RT, it is essential to maintain function using vigorous postoperative rehabilitation therapy supervised by a musculoskeletal oncology team. This is even more important in the skeletally immature child, where associated long-term complications, such as fibrosis and a painful or shortened extremity, should be avoided. Good to excellent results have been reported in 75% to 80% of adults, with more than 75% returning to full employment. At St. Jude, long-term complications include shortening, flexion deformity, chronic edema, fracture, and secondary malignancy noted in 7 of 50 patients with extremity NRSTS.[81,85]

### Surgery for Trunk Primaries

The primary incisional biopsy of trunk NRSTS is of paramount importance.[88,89 and 90] In most instances, biopsies should be in the longitudinal axis or parallel to the ribs. In the paravertebral area a vertical incision is preferred. Preoperative imaging studies determine the course and extent of resection needed to obtain satisfactory margins. It is important to recognize that the crucial aspect is the deep margin. If there is infiltration of the muscles or intrathoracic extension, a formal chest wall resection is performed. The anterior and posterior extent of rib resection is usually approximately 2.5 cm beyond the margin of the tumor. The superior and inferior extent is generally assumed to be one rib above or below the extent of primary lesion. The resultant deficit is usually closed with a prosthetic mesh or by regional muscle flaps. In young children and in situations in which extensive chest wall resection may predispose to severe scoliosis, consideration can be given to resection along the periosteum and placement of afterloading catheters for BRT.

### Surgery for Abdominal Wall Tumors

The abdominal wall is an unusual site for primary NRSTS. Accurate preoperative imaging studies are necessary to determine the extent of resection.[74,89] The entire thickness of the abdominal wall including the peritoneum, if necessary, can be resected. The major problem after resection of the peritoneum is that direct contact between intestine and nonabsorbable mesh used to close the defect has been noted to increase the risk of fistulization. To prevent this, an omental patch or placement of absorbable mesh is preferred. Local and regional rotation flaps may also be used to cover the defect. When margins are close, RT is considered for adequate local control. To minimize the effect of RT on the small bowel, polyglactin 910 (Vicryl) mesh is used to displace the bowel away from the radiation field.

### Surgery for Visceral Sarcomas

Visceral sarcomas are extremely rare. The most common variant is the gastrointestinal stromal tumor (formerly called *leiomyosarcoma*). The common sites include the large bowel and the stomach. Wide resection is the treatment of choice.[91] Failures, either local or metastatic, particularly to the liver, are associated with grade 3 tumor histology.

### Surgery for Retroperitoneal Tumors

Retroperitoneal sarcomas also are uncommon in children. Primary resection is the treatment of choice. In adult series, resection of the viscera including the kidney has been associated with a lower recurrence rate. Unfortunately, no large series are available in the pediatric age group. The morbidity of aggressive resection has to be weighed against the long-term risk of RT on viscera and bowel.

### Surgery for Head and Neck Sarcomas

Aggressive surgical resection is the mainstay for local control of head and neck sarcomas.[92] Modern reconstructive techniques, including locoregional rotation flaps, vascularized flaps, or free composite grafts have greatly assisted in the surgical resection. Local recurrences have been resected from the base of the skull or from the anterior skull using craniofacial exposure.

## RADIATION THERAPY

RT is an integral component of the treatment of NRSTS in children. The indications for RT vary according to tumor histiotype, pathologic grade, tumor size, location, extent of resection, and other clinical factors, including the overall treatment plan.[59]

After the indications for RT have been established, the best RT treatment modality must be selected. EBRT delivered by using a linear accelerator is the most commonly used RT modality. A variety of recently developed EBRT techniques are available for treating the pediatric patient. These techniques may be broadly described as *conformal RT*, in which three-dimensional imaging and treatment planning are used to minimize the risk to normal tissue structures that might be vulnerable to radiation.[93] BRT is the interstitial, intracavity, or superficial application of radioisotopes in a temporary or permanent fashion. Most commonly, iridium-192 and iodine-125 are used in the form of a low-dose-rate temporary interstitial implant that delivers approximately 35 to 40 cGy per min. BRT involves additional logistical considerations and requires a team with expertise in the treatment of NRSTS.[94] Newer forms of BRT that are available include the use of high-dose-rate sources that deliver treatment in a pulse fashion not unlike that of a linear accelerator.[95] Moreover, EBRT and BRT may be combined in a manner that optimizes the probability of local control and decreases the acute and late effects of therapy. When RT is indicated, decisions regarding the timing of RT, the total dose, the fractionated dose, and the treatment volume must be considered. All of these factors greatly influence the risk of side effects. At the center of this discussion is the selection of the appropriate treatment volume. Treatment volume critically influences local tumor control and treatment-related side effects. The use of established radiation delivery techniques such as BRT and the evaluation of new modalities such as conformal RT can improve the therapeutic value of RT for children.

### Historical Perspective

Suit and colleagues[96,97,98 and 99] showed in a series of reports that local control of NRSTS could be achieved with a high rate of success by combining surgery and RT. The reports outlined many of the technical and radiotherapeutic aspects of sarcoma management that remain in use to this day, including the use and necessity of conventionally fractionated RT (180 to 200 cGy per day) at a relatively high total dose (greater than 64 Gy) and a more formal definition of the treatment volume. Using the “shrinking field” technique, they treated a larger volume to a lower dose (50 Gy) and used a boost dose to deliver the highest or final prescription dose to a smaller volume. They carefully studied the planned use of EBRT or BRT as the boost modality, the sequencing of surgery and RT, including preoperative RT, and measures that could be used to minimize side effects and improve functional outcome.

Since the performance of this pioneering work, more wide local excision followed by RT has been a standard therapy in many cases for local control of NRSTS amenable to resection. Treatment approaches to adult NRSTS of the extremity have continued to change over the last 20 years. Modern limb-sparing surgery combined with RT has been shown to yield local control rates ranging from 85% to 95% while preserving limb function. Many treatment approaches that integrate limb-sparing surgery and RT include preoperative or postoperative EBRT with or without the use of BRT or intraoperative RT. The use of these methods in children and adolescents requires careful planning with an experienced pediatric radiation oncologist. To date, no clinical trial has compared these combined-modality approaches with respect to treatment results and functional outcome. Further, the data describing functional outcome in adults and children are limited.[100,101 and 102]

### Current Management Principles and Controversies

Early involvement of the radiation oncologist is important to the appropriate management of known or suspected NRSTS. If tumor control requires RT, the radiation

oncologist can assist the multidisciplinary team by defining the region to be treated and by considering function-sparing treatment using special BRT or EBRT modalities. Taking measures to delineate the extent of disease and to limit the irradiated volume can significantly reduce potential side effects. In an effort to standardize treatment and integrate the efforts of all members of the sarcoma team, consensus guidelines have been developed for the management of soft tissue sarcomas in adults.[103] Most of these guidelines are also applicable to children. Consensus guidelines have also been developed by the American Brachytherapy Society for the use of BRT in the treatment of adults and children with soft tissue sarcoma.[104]

Tumor location has important implications for both surgery and RT and plays an important role in the potential morbidity of RT. The need for maximal surgical resection and adjuvant RT for certain low-grade, most high-grade, and selected recurrent tumors is well accepted. Regardless of the extent of resection, however, the dose of radiation required to achieve local control (greater than 64 Gy) is relatively high when compared to doses used for the treatment of other pediatric sarcomas, rhabdomyosarcoma, and Ewing's sarcoma family tumors.

Surgical technique plays an important role in defining the treatment volume. When biopsy or resection of a soft tissue lesion is being planned, the radiation oncologist should be consulted by the surgeons to help plan an approach that avoids disruption of normal tissues and places the scar over the tumor. Placement of radiopaque clips to define the tumor bed or regions at highest risk will help with RT treatment planning. When resection is attempted, it should be performed with the objective of achieving negative margins, good hemostasis, and a good functional outcome. Incisional biopsy should be considered when resection is likely to be incomplete. However, children are often referred for evaluation and treatment after the incomplete resection of an NRSTS that was thought to be a benign lesion. Although there is poorer outcome for patients treated without a comprehensive multidisciplinary plan, high-dose RT can be used in such cases in an adult to achieve local control. When resection is not possible, advanced RT techniques are beneficial; however, the local failure rate and local morbidity are higher.

RT may be avoided for selected patients with high-grade NRSTS who have undergone wide local excision with the removal of a large cuff of normal tissue and documented negative biopsies of the surgical cavity. It would also be reasonable to identify subsets of patients who do not require irradiation and who should not be subjected to its long-term morbidity.[105] The following presentations may not require radiation: histologic margins of resection of greater than 1 cm, and a local recurrence in a site that does not preclude function-sparing surgery. Although we have found that the tumors in children are relatively smaller than those found in adults, the type of wide local excision that is likely to obviate the need for RT is also likely to cause substantial surgical morbidity. Furthermore, a generous wide local excision is often impossible in children because of their lack of subcutaneous tissue. Many NRSTSs have satellite nodules within the tumor bed, even when the inked margins are negative. When a non-oncologic surgical procedure is performed, RT is virtually always required. There are limited data on the indication for radiation based on the extent of the surgical margin in children with NRSTS. In one small study of high-grade tumors with resection margins less than 1 cm, successful local control was achieved in five of seven patients not treated with RT and in seven of seven patients when RT was given postoperatively. Of 20 high-grade tumors that were completely resected with margins greater than 1 cm and were not treated with adjuvant RT, 15 were locally controlled for an extended period.[106] More data are required to define clearly the role of RT based on resection margin. Despite this, high-grade tumors that are completely resected with close margins often are treated with EBRT or BRT to enhance local control. BRT may be combined with EBRT for high-grade and intermediate-grade tumors with positive, inadequate, or indeterminate margins, without regard to tumor size or anatomic location.[94] Low-grade tumors are treated with EBRT or BRT only when the risk of recurrence and reresection morbidity is high, or when there has been a recurrence. RT may be avoided for small, superficial tumors in very young patients when resection can be performed with adequate margins, generally greater than 5 mm. Aggressive multimodality therapy including RT may enhance local control and quality of survival in patients with metastatic disease, as well.

### Importance of Local Control

Despite the high rate of success in achieving local control, optimal disease-free survival and survival for pediatric NRSTS has not yet been achieved.[10] Distant metastases are most likely a reflection of aggressive tumor biology rather than local treatment approaches. Thus, improved systemic strategies are required to improve survival; however, because long-term survival cannot be achieved without local tumor control and because tumor recurrence and second surgery cause substantial morbidity, every effort should be made to achieve local control with the least morbidity and smallest impact on quality of life. Unfortunately, insufficient numbers of children have been studied to determine the effect of local control measures on survival.

### Differences between Adults and Children with Soft Tissue Sarcoma

The treatment of soft tissue sarcomas in children requires sophisticated methods of RT treatment planning and delivery to improve the therapeutic ratio of RT. Indeed, children have the most to gain from advances in RT that reduce late effects. Because high doses of radiation are necessary to achieve local control of NRSTS and because many patients with NRSTS are at an age that makes them especially susceptible to devastating side effects, children with NRSTS should be referred to treatment centers equipped with advanced treatment delivery techniques and experience in treating children. However, there is no evidence that these tumors are more sensitive to RT in children than when they occur in adults. Thus, the use of lower doses in an effort to limit side effects cannot be recommended outside of carefully constructed and controlled clinical trials.

### Radiation Therapy Planning

Advanced methods of EBRT treatment planning and delivery have been developed to ensure that the prescribed radiation dose conforms to well-defined targets and spares normal tissues from high doses of irradiation. These methods have been adopted to increase the accuracy of RT, decrease the potential for late effects, and improve local control through improved targeting and dose escalation. These two methods, conformal RT and BRT, may be used alone or in combination when indicated for local control.[93]

RT for NRSTS requires experience and precision. A planning computed tomography (CT) scan of the extremity is mandatory, and treatment facilities should have the capability to use both CT and MRI for the planning process. Correlating the tumor volume as defined by the preoperative MRI study and the studies used in the RT planning process is necessary. A preoperative dose of 45 to 50 Gy or a postoperative dose of 60 to 65 Gy (conventional fractionation) is commonly prescribed and is administered by using a shrinking field technique. The initial field should encompass all areas of gross disease, as defined by preoperative MRI, or the tumor bed, as defined by the surgical clips. When possible, the initial field is usually treated with 5-cm margins; however, treatment of the entire cross section of the extremity is avoided to prevent edema and loss of function. Immobilization of the extremity with customized molds is an important component of radiation treatment planning.

Advanced methods of treatment planning and delivery may offer great potential benefits to children with NRSTS. It has become increasingly apparent that wide treatment margins (greater than 5 to 10 cm) and inclusion of the scar and drain sites, formerly advocated for all patients, are not necessary when strict surgical oncology practices are applied and intraoperative assessment of the tumor is performed.[107] Indeed, the results of a prospective randomized trial of adjuvant BRT for extremity and truncal soft tissue sarcomas in adults suggest that margins of 2 cm are adequate.[102] In that study, the target area for BRT was defined by adding a 2-cm margin beyond the superior and inferior extent of the tumor bed and 1.5 to 2.0 cm laterally. The rate of local control was 82% for adult patients with high-grade sarcomas treated in this manner without EBRT.[108]

A simplified prescription for RT includes a stated dose and volume. The two are melded into a physical representation of the treatment. *Three-dimensional conformal RT* is any method that is capable of conforming (shaping) the isodose distribution to the target in three dimensions. The value of a particular conformal treatment plan is determined by its reproducibility, the percentage of the planning target volume that receives the prescription dose, and the nominal dose to normal or critical structures. Such a treatment plan is currently accomplished by immobilizing the patient in a manner that is reproducible for daily treatment. Immobilization plays an important role in realizing a conformal treatment plan. Once a patient is adequately and reproducibly immobilized, CT or MRI data are obtained with the patient in the treatment position. The image data are then transferred to a planning computer, after which specific targets are defined and dose-limiting structures or normal tissues are identified within the image data. Most institutions adhere to the International Commission on Radiation Units definitions of the tumor volumes.[109] These include the gross tumor volume, clinical target volume, and planning target volume. The gross tumor volume includes any gross tumor or the tumor bed. The clinical target volume generally includes the gross tumor volume with an anatomically defined margin meant to treat subclinical microscopic disease; the margin is selected according to tumor type, anatomic site, and pertinent clinical-pathologic information relevant to the patient's overall treatment plan. A geometric margin is added to the clinical target volume to create the planning target volume. This additional margin is meant to consider all possible geometric variations and daily variability in localization. With the above-mentioned targets defined, the radiation beams are oriented to achieve trajectories that minimize the dose delivered to normal tissues; this process results in a conformal treatment plan. Expert judgment is required to balance the desire to achieve a conformal isodose distribution that decreases sharply outside of the planning target volume with the possibility of irradiating larger volumes of normal tissue with low doses of radiation. When developing a conformal treatment plan, one should not limit the orientation of the beams to a single plane (coplanar), which will substantially limit the ability of the treatment to conform to the target volume. The beams should be allowed to approach the target volume from any orientation (noncoplanar) and should preferably traverse the least amount of normal tissue or traverse normal tissues that are least susceptible to radiation effects. The entrance and exit characteristics of the radiation beams greatly influence the irradiated

volume. Finally, the orientation and number of radiation beams should be chosen to obtain a treatment plan that adheres to institutional definitions of conformality and safety. Under certain circumstances, the beam arrangement may resemble that used in a conventional treatment plan. [93]

### Preoperative Radiation Therapy

RT given before surgical resection has a number of therapeutic and functional advantages for all types of NRSTS. Therapeutically, preoperative RT treats a nonhypoxic tumor bed, allows limited-volume irradiation, improves resectability, and lessens the likelihood of intraoperative contamination. Functionally, preoperative RT can preserve organs or neurovascular structures, epiphyseal plates, and physiologic function either through limited-volume RT using conformal techniques or by permitting limited resection. There is a good radiobiologic rationale for preoperative RT. Even with a large tumor mass, preoperative RT to a dose of 45 to 50 Gy may inactivate more than 99% of the tumor cells. This may decrease the likelihood of tumor implantation during surgery. Tumor cells that are shed into the circulation at the time of surgery are also hypothetically less likely to be viable or able to establish distant metastases. With preoperative RT, the radiation oncologist need treat only tissues thought to be at risk for tumor involvement; the radiation fields need not encompass areas to be manipulated by the surgeon, as is the case in postoperative therapy. The greatest advantage to the use of preoperative RT may be that it allows some patients to undergo limb-salvage procedures rather than amputation. Even if the tumor mass does not decrease in size after RT, the lesion may consist of necrotic debris with little viable tumor. A potential difficulty with preoperative RT is delayed healing of the surgical wound, although if good surgical and radiotherapeutic techniques are used, primary healing should occur. There are several series that demonstrate that planned preoperative RT in patients with high-grade sarcoma can yield excellent results, with 5-year disease-free survival rates of 56% to 74% and local control rates of 83% to 95%. [97,110,111] Wound complication rates of 6.5% to 30%, similar to or exceeding those that would be expected with postoperative RT, have been reported with conventional methods of treatment planning and appear to be the only negative aspect of preoperative RT. [100,112] The greatest gains with preoperative treatment have been shown in the treatment of large tumors [99,113] and in studies combining preoperative RT with preoperative chemotherapy. [114] The general guidelines for preoperative RT of NRSTS include EBRT to 50 Gy; resection 3 to 4 weeks later; and a boost to 64 to 68 Gy using intra- or postoperative EBRT, intraoperative high-dose BRT (Ir-192), or low-dose BRT (I-125 or Ir-192).

The experience of preoperative RT for pediatric NRSTS is limited. Because it may be possible to use lower doses of radiation when RT is combined with surgical excision in this manner, studies that define the optimal sequencing of RT and surgery are needed. The National Cancer Institute of Canada is conducting a phase III trial of preoperative versus postoperative EBRT in curable adult NRSTS of the extremity. [115]

### Postoperative Radiotherapy

There are several theoretical advantages of postoperative RT. These include the institution of immediate surgery, avoidance of radiation-induced delays in wound healing, and the availability of a large specimen for histopathologic investigation with definitive interpretation of tumor size and extension. With this approach, the initial postoperative treatment volume should include the entire wound, which usually is larger than the volume that would have been treated preoperatively. Several retrospective, single-institution series have assessed this approach. The 5-year results include a disease-free survival rate of 60% to 68% and a local control rate of 78% to 92%. [116,117 and 118] In the late 1970s and early 1980s, the NCI conducted a small randomized, prospective trial to compare the results of limb-sparing surgery with postoperative RT to amputation in patients with high-grade sarcomas of the extremity. [65] Although the local control rate was slightly better with amputation, the groups had similar disease-free and overall survival rates. In the limb-sparing group, 85% of patients retained a functional limb. Clearly, the functional preservation of the extremity is an important theoretical advantage of conservative surgery and postoperative RT as compared with radical resection or amputation. Little objective information has been available, however. Some small, retrospective studies have found no quality of life advantage in limb salvage as compared with radical treatments. [119,120] These results are difficult to understand and are counterintuitive. In 1991, Stinson and colleagues [101] from the NCI reported the long-term effects of conservative surgery and irradiation on functional outcome in 145 adult patients with sarcoma of the extremity who were treated between 1975 and 1986. Overall, 84% of patients were ambulating without an assistive device and with mild or no pain. As more precise RT methods evolved at the NCI over the 11-year period, the complication rate decreased. Although the extent of surgery clearly influenced functional outcome, the concomitant use of adjuvant chemotherapy, including doxorubicin, did not.

Two more recent NCI trials have assessed the role of postoperative RT for adult soft tissue sarcomas of the extremity. Ninety adults with high-grade (histologic grade 2 or 3 on a scale of 1 to 3) lesions were randomly assigned to receive either adjuvant postoperative irradiation (45 Gy for 5 weeks to the wide surgical bed, with an 18-Gy boost over 2 weeks) and adjuvant postoperative chemotherapy or adjuvant postoperative chemotherapy alone. [121] With a median follow-up of 5 years, none (0%) of 44 patients in the RT group had local treatment failure, whereas 9 (20%) of 46 patients had local failure after surgery and postoperative adjuvant chemotherapy alone ($p = .002$). However, there was no difference between the two groups in the rate of 5-year survival (80%). In the second trial, [60] patients with low-grade (grade 1) NRSTS of the extremity were randomly assigned either to receive postoperative irradiation (doses similar to those used in the previous trial) or to be observed after wide resection. [122] With a median follow-up of 8 years, there have been six (21%) local failures among 29 patients under observation and one local failure among 28 patients receiving postoperative RT ( $p = .06$). The single local recurrence in the adjuvant RT group occurred at the margin of the radiation field. Overall, eight patients on the observation arm have developed recurrent tumor, as compared to three patients who received RT ( $p2 = .08$). Although these groups may have been too small to allow the detection of a statistically significant difference in the rate of recurrence, postoperative RT appears to be indicated even for patients with low-grade sarcomas. [122]

### Brachytherapy

BRT is the intracavitary, interstitial, or surface application of radioisotopes or radiation-generating devices; they may be permanent or temporary. BRT is an excellent alternative or adjunct to EBRT. It has inherent tissue-sparing properties and dosimetric advantages that can be used to deliver RT in a conformal manner. [123] The technique relies heavily on preimplant assessment of tumor extent, including biopsy of the surgical field, diagnostic imaging, and the extent of resection. In general, the implant volume is limited to the tissues exposed at the time of surgery, although with careful technique, catheters may be placed approximately 2 to 3 cm beyond the wound. Temporary implants, the most commonly used form of BRT for pediatric patients, are readily placed in most head and neck, truncal, extremity, and intracavitary sites. In the administration of low-dose BRT, for which there is a reasonable pediatric experience, catheters or guiding devices are placed at the time of surgery and are loaded with radioactive sources 5 to 6 days after the operation. These sources remain in place for a prescribed interval of time and are removed together with the catheters or guiding devices. Permanent implants are not commonly used in the primary management of pediatric sarcomas. They are reserved for situations in which normal tissue tolerance has been exceeded with EBRT and high doses of localized RT may be effective.

BRT is an excellent treatment option for pediatric patients with NRSTS. Disease control may be achieved with a high rate of success when BRT is used alone or in combination with EBRT. This treatment approach offers several advantages over EBRT for pediatric patients with NRSTS reducing the dose of radiation to normal tissues and shortening the overall treatment time while maintaining a comparable rate of local control. The reduction of radiation doses delivered to normal tissues decreases the probability of growth deformity, adiochemotherapy interactions, and hypothetically, the risk of second tumor formation.

The value of BRT for NRSTS has been consistently demonstrated in adults. [108,124,125 and 126] An initial series of 33 patients with locally advanced sarcomas, many of which would have required amputation, resulted in an 88% local control rate. [100] The rate of local control was 70% for patients with high-risk tumors that abutted or actually invaded a major neurovascular bundle, which had been dissected and preserved. Subsequently, a prospective randomized trial at Memorial Sloan-Kettering Cancer Center confirmed the highly significant effect of BRT in preventing local recurrence after limb salvage surgery. [108,127] Local failures occurred in 2 of 52 patients randomly assigned to receive BRT and in 9 of 65 patients who did not receive BRT ( $p = .06$). The results were more significant in an analysis of patients with high-grade lesions, in which none of the 41 patients who received BRT had local failure, but 5 of 47 patients who did not receive BRT ( $p = .03$) had local failure. The most common major complication of BRT was poor wound healing. Improvements in surgical technique and standardization of the radiation treatment planning, including a planned delay of loading for at least 5 days after surgery, have reduced the rate of wound complications. There is also evidence that adult patients with involved surgical margins have a higher probability of local control of local disease when treated with combined BRT and EBRT than when treated with implants alone. [124] In children, the data are more limited; the reported series include relatively small numbers of patients with different tumor types. [94,128,129,130,131 and 132] The advantage of BRT over wide local excision alone for local control was conclusively demonstrated in adults with high-grade tumors. [108] Because assessments of BRT in children include patients with rhabdomyosarcoma and Ewing's sarcoma family tumors, there are limited data specific to NRSTS in children. Thirty-one children with NRSTS who were treated with BRT were reviewed at St. Jude Children's Research Hospital. Twenty-seven patients were initially managed with BRT; ten of these patients had BRT alone, and the remainder were treated with a combination of BRT and EBRT. There was no local or regional treatment failure in the group treated with BRT alone. Eight patients were alive with no evidence of disease 9 to135 months after diagnosis. There was one local failure in the BRT plus EBRT group. [94]

Although BRT considerably shortens the overall treatment time and has local control rates that approach those reported for conventional preoperative or postoperative EBRT, we recommend its application only at major cancer centers with active BRT services. Prospective trials of BRT for children with soft tissue

sarcomas are needed.

### Intraoperative High-Dose Rate or Electron Beam Radiation Therapy

Intraoperative high-dose rate (IOHDR) RT or electron beam RT (IORT) is the application of BRT or EBRT at the time of operative exposure of the treatment volume.[133,134] The major advantage of IOHDR and IORT is the opportunity to remove, reposition, or shield critical structures such that high-dose, single-fraction RT can be precisely delivered to the tumor bed with minimal toxicity. These advantages improve the therapeutic ratio of the treatment and often allow RT to be given in a location in which normal tissues would be damaged if they were traversed by EBRT. This is especially true for previously irradiated locations. IOHDR or IORT can be given instead of or as a boost treatment before or after EBRT. In this technique, a tissue-equivalent material containing patent catheters is positioned in the operative bed. To irradiate the target, the catheters are connected to a remote afterloader and the source is then conveyed from the afterloader into the catheters, in which it dwells at defined positions for prescribed time intervals. Sites that are difficult to reach are treated by this method. A specially shielded operating room is required, and the patient is remotely monitored during the treatment. The use of 1,200-cGy single-fraction IOHDR in 16 pediatric patients with solid tumors, 11 of whom had bone or soft tissue sarcomas in abdominal, thoracic, or pelvic sites, resulted in actuarial rates of local control, metastasis-free survival, and overall survival of 61%, 51%, and 54%, respectively, with a median follow-up of 18 months.[134] These patients' tumors would be classified as high risk on the basis of their clinical presentation. The outcomes we observed in this small cohort suggest that IOHDR is a safe method for delivering RT and for escalating the total dose of RT.

### Nonextremity Soft Tissue Sarcomas and Special Sites

Approximately 40% of soft tissue sarcomas occur in nonextremity sites such as the retroperitoneum. The surgical approach to these tumors is constrained by the lack of feasibility of wide local resection. Adjacent vital structures often limit surgery to local excision with, at best, limited margins of resection. Because of the difficulty of obtaining wide, negative margins, postoperative RT is usually recommended. Intraoperative RT and dose escalation has been tested most commonly in the retroperitoneum.[135,136 and 137] Sarcomas as a group are not common tumors, and NRSTSs arising in the hand and foot are very rare. Unlike alveolar rhabdomyosarcoma, most patients with NRSTS of the hand or foot, excluding epithelioid sarcoma, have localized disease at presentation and may be at lower risk for developing metastases, probably because the tumors are smaller and are detected earlier. Given the functional complexity of the hand and foot and the better prognosis for this patient group, the issue of how best to establish local control and maintain function is important. Previously, it was assumed that the high doses of radiation required to control even microscopic sarcoma would result in such poor function of the hand or foot that amputation was considered the treatment of choice. It is now apparent that this assumption was wrong; two series have documented excellent tumor control with preservation of function. [138,139]

### Acute and Long-Term Sequelae of Radiation Therapy

The most common side effects of EBRT or BRT—radiation dermatitis, cellulitis, fibrosis, and telangiectasia—are reported to occur in 10% to 50% of cases. Radiation osteitis may be seen.[140] Second malignant neoplasms may also be induced by RT.[93] The effects on the musculoskeletal system in young and growing children require careful treatment planning by experienced pediatric radiation oncologists. These effects include limitation of growth of bones and soft tissues. This may lead to leg length inequity and gait disturbances. Joint dysfunction with decrease in range of motion may also contribute to gait disturbance. Reduction of muscle mass with the radiation field may result in weakness. Bone fracture may occur as a consequence of RT, especially when the surrounding musculature is reduced due to surgery, radiation, or both. Circulatory complications and resulting edema may also be seen.

### Conclusions

RT remains one of the most effective primary and adjuvant treatments for pediatric sarcomas. When RT is indicated, treatment is usually focal, and attempts are made to spare normal tissues as much as possible without reducing the probability of local control. Because of this consideration, BRT and conformal methods of RT treatment planning and delivery should be considered for pediatric patients with NRSTS.

## CHEMOTHERAPY

### Adjuvant Therapy—Adult Trials

The role of adjuvant chemotherapy in the treatment of adult NRSTS continues to be controversial. To date, only 3 of 15 randomized adjuvant chemotherapy trials in adults have shown a significant survival advantage for the adjuvant chemotherapy group. [141,142] The first study randomized 59 patients (36 with extremity primary tumors) to receive 8 to 11 courses of cyclophosphamide, vincristine, dacarbazine (DTIC), and doxorubicin (CYVADIC) versus observation. Five-year metastasis-free survival and overall survival were significantly better for the chemotherapy group: 65% versus 34% ( $p = .003$) and 83% versus 43% ($p = .002$), respectively. A second study randomized 77 patients with high-grade extremity NRSTS to observation versus single-agent doxorubicin and found a significant survival and disease-free survival advantage for patients who received adjuvant chemotherapy.[141] A third study randomized 104 patients with high-grade, large, primary extremity soft tissue sarcomas to chemotherapy using the combination of epirubicin and ifosfamide versus observation. At a median follow-up of 36 months, patients randomized to the chemotherapy arm had significantly higher overall and disease-free survival estimates. [142] All of the remaining randomized studies of adults showed no benefit of adjuvant chemotherapy. Thus, the benefit of adjuvant chemotherapy has not been clearly established.

Several additional adult trials have shown improved disease-free survival when adjuvant chemotherapy is used. The first, a small randomized study of adjuvant chemotherapy in 65 adults with NRSTS of the extremities at the NCI showed a disease-free survival benefit for patients who received adjuvant chemotherapy with cyclophosphamide and doxorubicin.[65] With a median follow-up of 7.1 years, the 5-year disease-free survival was 75% for those treated with chemotherapy and 54% for those receiving no chemotherapy.[65,143] This result was not duplicated in the NCI series for patients with sarcomas of the trunk. The second series of adult patients observed for more than 10 years at M. D. Anderson Hospital showed the disease-free survival rate of those patients treated with vincristine, Adriamycin, cyclophosphamide, dactinomycin, and irradiation was 55%, compared with 35% ($p = .05$) for patients treated with surgery and irradiation alone. [144] The third study conducted by the European Organization for Research on the Treatment of Cancer (EORTC) demonstrated higher relapse-free survival rates in patients who received adjuvant chemotherapy; however, no survival advantage for the chemotherapy arm was evident.[145] In contrast, four randomized trials evaluating the role of Adriamycin in the adjuvant treatment of NRSTS did not reveal any clear benefit. [146,147,148 and 149]

Of interest, meta-analysis of 14 randomized trials of adjuvant chemotherapy comprising 1,568 adult patients with NRSTS demonstrates a reduction in the risk of local failures and distant failures at 10 years with a 10% recurrence-free survival and 4% survival advantage for those receiving adjuvant chemotherapy with doxorubicin-based regimens.[145a] Extremities have the clearest evidence of treatment effect. These data show only a minimal benefit in adult patients with NRSTS.

### Adjuvant Therapy—Pediatric Trials

The role of adjuvant chemotherapy in the treatment of pediatric rhabdomyosarcoma has been well established; however, its role in the treatment of NRSTS remains controversial. In children, there has only been one prospective randomized trial of adjuvant chemotherapy for NRSTS. From June 1986 through May 1991, the POG evaluated the benefit of adjuvant chemotherapy (vincristine, Adriamycin, cyclophosphamide, and dactinomycin) compared to observation in surgically resected pediatric NRSTS.[61] Of the 81 eligible patients, only 30 accepted randomization and no evidence of improved outcome could be seen in the subgroup that received adjuvant chemotherapy. Five-year survival and event-free survival estimates were significantly worse for patients who received adjuvant chemotherapy; however, this is likely the result of an imbalance in the number of patients with high-grade lesions who received adjuvant chemotherapy. Because an adult study suggests that ifosfamide may be more effective than cyclophosphamide against these sarcomas, future randomized trials of adjuvant chemotherapy in pediatrics will likely use this agent in combination with anthracyclines.[150,151 and 152]

Based on existing data in children, the role for adjuvant chemotherapy for surgically resected NRSTS has not been established. Its use should continue to be investigated in the context of multi-institutional trials. Patients with extremity primaries and close margins may benefit from adjuvant chemotherapy to reduce the risk of local recurrence; however, this will require careful evaluation.

### Therapy for Advanced Disease—Adult Trials

There are a number of active regimens in the treatment of advanced NRSTS: MAID (mesna, Adriamycin, ifosfamide, and DTIC),[153] MAP (mitomycin C, Adriamycin,

and cisplatin),[154] CYVADIC,[155] Adriamycin,and cyclophosphamide with or without vincristine, [64] and ifosfamide with etoposide.[156,157] All of these regimens have a significant response rate in advanced disease.

In a series of trials of adults with advanced soft tissue sarcomas, the EORTC has demonstrated the following: that (a) DTIC as part of CYVADIC did not add to the outcome seen with Adriamycin plus cyclophosphamide; (b) ifosfamide is preferable to cyclophosphamide because it results in a higher response rate, equivalent survival, and less toxicity when given as a single agent; (c) ifosfamide is active in some cyclophosphamide-resistant tumors; (d) addition of ifosfamide to an Adriamycin regimen results in a higher response rate; (e) on the basis of sequential trials, the dose of Adriamycin is apparently directly related to the response rate; and (f) higher doses of Adriamycin are associated with a prolonged disease-free interval. [158]

One of the trials, an Intergroup Phase III randomized study of doxorubicin and dacarbazine with or without ifosfamide and mesna in 340 adults with advanced soft tissue and bone sarcomas demonstrated a longer time to progression, but more myelosuppression after ifosfamide and mesna were seen. In this study and in two additional randomized trials of doxorubicin (Eastern Cooperative Oncology Group and EORTC) with and without ifosfamide, the response rate was higher in the ifosfamide-containing arms.[159] Another recent EORTC trial randomized 294 eligible patients to receive standard-dose doxorubicin (50 mg per $m^2$ on day 1) and ifosfamide (5 g per $m^2$ on day 1) versus an intensified regimen combining doxorubicin (75 mg per $m^2$ on day 1), with the same ifosfamide dose followed by granulocyte-macrophage colony-stimulating factor.[145] Objective responses were observed in 21% of patients in the standard arm and in 23% of patients in the intensified arm. Progression-free survival was significantly longer in the intensive arm, but there was no difference in overall survival between the two therapeutic arms.

### Therapy for Advanced Disease—Pediatric Trials

Pediatric studies in advanced NRSTS are limited. A recently reported POG study evaluated the role of DTIC in the context of multi-agent chemotherapy with vincristine, Adriamycin, cyclophosphamide, and dactinomycin for patients with unresected or metastatic NRSTS. [61] The addition of DTIC did not improve outcome, and the overall results were similar to those reported in adults: overall survival and event-free survival at 4 years were 30% and 18%, respectively. The POG has recently completed a pilot study of vincristine, ifosfamide, and doxorubicin for similar patients. The results of this trial are not yet available. Although many chemotherapy regimens have activity in advanced NRSTS in both children and adults, significant improvement in survival for children has not been achieved with the current regimens. Based on adult trials from the EORTC, more intensive chemotherapy regimens that use higher doses of ifosfamide and Adriamycin may be beneficial in the treatment of advanced NRSTS. Few data exist as yet in the treatment of children, however. The described pilot POG trial will contribute to our understanding.

### Conclusions

The most important issues to address in the chemotherapy of children with NRSTS are the following: (a) to discover very effective therapy for children with NRSTS; (b) to define the role of adjuvant chemotherapy in children with surgically excised tumors; (c) to evaluate the impact of chemotherapy on children who present with metastatic disease or with unresectable nonmetastatic tumors; (d) to determine the role of ifosfamide in combination with Adriamycin or etoposide in the treatment of children with NRSTS, especially those with advanced disease; (e) to evaluate the optimal dose intensity of the chemotherapy agents and regimens used to treat NRSTS.

## CONGENITAL FIBROSARCOMA

### Epidemiology, Biology, and Genetics

Although rare, fibrosarcoma is one of the most common NRSTSs in children and adolescents.[160] It is the most common soft tissue sarcoma occurring in children younger than 1 year.[1,40,160] Historical series of patients with congenital fibrosarcomas suggest that there is a predominance of boys [40,41]; however, another report and review of the literature reveal no significant gender predominance. [42] There appear to be two peaks in incidence of fibrosarcoma according to age: in infants and children younger than 5 years, and in patients 10 to 15 years of age. Although some authors believe that the biology of these tumors is similar in the two age groups, most think that the tumors in infant congenital fibrosarcomas usually have a more benign course. [40,41,161] Despite its name, this is not a fibrosarcoma in the conventional sense, and it does not commonly metastasize despite repeated local recurrences. [40] The Armed Forces Institute of Pathology (AFIP) series document a low metastatic rate of 10%. In fact, this low level of metastatic potential, combined with biologic observations such as a failure of xenograft tumor formation in mice and a near-diploid karyotype on flow cytometry have led some to question the terminology “fibrosarcoma.” [162,163] However, careful karyotypic studies have clearly documented a recurring t(12;15) translocation, which has been cloned recently, revealing a novel fusion gene formed by ETV6 (or TEL) with the high-affinity nerve growth factor NTRK3 (TRKC). This abnormality is not found in infantile myofibromatosis or other early childhood fibromatosis, nor is it found in adult-type fibrosarcomas occurring in older children. [164,165] This unique abnormality clearly distinguishes this tumor from other, similar tumors such as infantile myofibromatosis and congenital hemangiopericytoma, with which it is sometimes confused.[166]

### Pathology

Histologically these tumors are composed of a uniform population of fibroblasts or myofibroblasts. Little stroma is present; when it is, it is often loose or poorly formed collagen, best seen on reticulin stain as opposed to trichrome stain. The mitotic rate is generally low but can be quite high, a finding of no prognostic significance. Retroperitoneal and head and neck lesions generally fare worse than extremity lesions despite an identical histology.

### Clinical Presentation

In a review of the literature detailing the experience with 52 cases of congenital fibrosarcoma, 37 were found to have occurred on an extremity and 15 on the trunk. [42] The presentation is usually with a localized mass with no systemic symptoms.

### Principles and Recommendations for Treatment

The usual treatment strategy for infants with congenital fibrosarcoma is surgical extirpation by wide local excision without additional radiation or chemotherapy. Of the patients with extremity primaries described above, 92% were free of metastatic disease and 95% were alive despite a 32% local recurrence rate; only six have died (11.5%).[42] Two children who died of recurrent congenital fibrosarcoma were reported to have had a pathologic pattern in the tumors that was typical for MFH at the time of local relapse.[167] This finding suggests a relation between fibrosarcoma and MFH that is not well recognized or understood. Because late local recurrences do not appear to affect overall survival of patients with congenital fibrosarcoma, conservative surgical management aimed at maintaining as much function as possible and avoiding amputation is often the preferred approach. Moreover, because of the known long-term consequences of RT and the potential late effects of chemotherapy, the use of these modalities in the local management of congenital fibrosarcoma is generally not advised unless surgical removal is not possible initially. A number of investigators have reported a dramatic reduction in size of large masses with the use of preoperative chemotherapy. [168] This approach has allowed more conservative surgical approaches without amputation. Chemotherapeutic regimens that have been used include ifosfamide, vincristine, and dactinomycin; vincristine, dactinomycin, and cyclophosphamide; and vincristine and dactinomycin without an alkylating agent. [169,170 and 171] Vincristineand dactinomycin are preferred initial agents because of their relative lack of long-term side effects and lack of leukemogenesis. There is no established role for adjuvant chemotherapy after a surgical procedure in the management of congenital fibrosarcoma.

For congenital fibrosarcomas that arise in axial locations, the local recurrence rate is similar to that for extremity tumors (33%); however, the metastatic rate and mortality rate are higher, 26%, compared with 10% or less for extremity primaries.[42,43]

## FIBROSARCOMA

### Epidemiology, Biology, and Genetics

Unlike congenital fibrosarcoma, conventional fibrosarcoma is a full-fledged malignancy with very real potential to metastasize, particularly when it occurs in older, especially postpubertal, patients. Although this diagnosis is infrequently made in adults, in the pediatric age group, this is a well-recognized tumor. Histologically, it is not different from fibrosarcomas in adults. The behavior is similar as well. Because virtually all of these tumors occur in postpubertal adolescents, age is a strong

predictor of both histology and prognosis among fibrous tumors in this age. Clonal translations t(X;18), t(2;5) and t(7;22) have been reported in adult-type fibrosarcoma.[172]

Laboratory studies of many malignancies have revealed characteristic cytogenetic and molecular genetic alterations that are useful in diagnosis or for prediction of clinical behavior (i.e., metastasis and treatment responsiveness). Recurrent genetic abnormalities have been noted in fibrosarcoma, [173] but a reliable method of distinguishing between high- and low-grade lesions is so far unknown. Because mutations in the p53 oncogene have been observed at high frequency in many soft tissue sarcomas,[174],[175],[176],[177],[178],[179] and [180] and because p53 mutations have been associated with poor prognosis within groups of similar tumors, [181],[182],[183] and [184] it may be suspected that p53 mutations could be used to discriminate true fibrosarcoma from other fibrous tumors. [185] However, this has not proved to be the case in larger series of tumors subjected to DNA sequence confirmation of p53 mutations. [186]

### Pathology

Fibrosarcoma is a spindle cell tumor with a characteristic herringbone pattern made up of regularly interweaving fascicles of parallel arrays of tumor cells ( Fig. 34-4A). Important features include evidence of abnormal mitoses, nuclear pleomorphism, and increased basophilia of individual, sometimes anaplastic, tumor cells. Cells are densely packed, but reticulin stain reveals a regular pattern of stromal collagen fibers not easily appreciated by light microscopy. The most important entities to distinguish are aggressive fibromatosis (which can be exceedingly aggressive locally but does not metastasize), nodular fascitis, myositis ossificans, and inflammatory pseudotumor among nonmalignant conditions, and neurofibrosarcoma, MPNST, poorly differentiated embryonal rhabdomyosarcoma, and monophasic (spindle cell) synoviosarcoma among malignant tumors (Table 34-10). It is not always possible to differentiate congenital fibrosarcoma from fibromatosis [41] and other fibrous tumors. Individual tumors must be evaluated in light of clinical information such as age, site, history, and duration of the lesion. In particular, fibrosarcoma in the first year of life is a serious but rarely metastasizing tumor; the apparently same tumor in an adolescent frequently develops metastases. [163] Therefore, age must be considered when making the diagnosis. Intermediate ages present challenges to pathologists and oncologists alike. Confusion with benign fibrous lesions such as myositis ossificans (a posttraumatic lesion) and nodular fascitis has occasionally led to overtreatment.

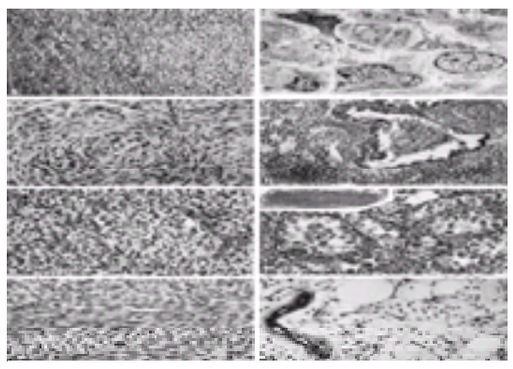

**FIGURE 34-4.** Differentiated soft tissue sarcomas other than rhabdomyosarcoma. **A:** Fibrosarcoma. This tumor is rare in adults and uncommon in older children, but it is one of the most common nonmyogenous soft tissue sarcomas in the first decade. Interlacing fascicles of spindle cells appear either elongate ( *left*) or round (*right*), depending on plane of section. The cells are closely packed and homogeneous, and resemble normal fibroblasts. Mitoses, pleomorphism, and nuclear hyperchromatism are rare [hematoxylin and eosin (H&E) stain, ×250]. **B:** Neurofibrosarcoma. The light microscopic appearance of this tumor may be indistinguishable from that of fibrosarcoma. Electron microscopy (EM) reveals evidence of nerve sheath differentiation, although not often as well developed as seen here, in which some tumor cells envelop neurites (*upper center*), mimicking Schwann cells. More often, only fragmented basal lamina and slender cell processes are present (EM, ×2,000). **C:** Malignant fibrous histiocytoma (MFH). The most common soft tissue sarcoma of adults, MFH is uncommon in children. The angiomatoid variant, seen here, is frequently seen in children. The propensity for tumor cells to form vascular structures ( *center*) accounts for the name (H&E, ×250). **D:** Synovial sarcoma. Classic biphasic synovial sarcoma is an unmistakable entity. Distinct epithelial, glandular differentiation ( *center*) alternates with fibrosarcomatous stroma (*right*). Islands of tumor cells separated by hyaline stroma are sometimes seen (*left*), and staghorn vasculature (not illustrated) is often conspicuous (H&E, ×100). **E:** Hemangiopericytoma. Thought to recapitulate normal pericytes, this tumor varies from Ewing's-like (as here) to a spindle cell variety resembling smooth muscle. In all cases, reticulin stains outline individual tumor cells and nests. EM demonstrates evidence of pericytic differentiation. Tumor cells do not form vascular spaces but surround them (*center*) (H&E, ×250). **F:** Alveolar soft part sarcoma. This problematic tumor is easily differentiated from all other sarcomas. The glandular or alveolar pattern is always prominent; tumor cells appear to rest on a basement membrane. Spaces within are variably present, as here. The tumor cells possess abundant pink cytoplasm that by light microscopy resembles muscle. However, periodic acid–Schiff–positive, diastase-resistant cytoplasmic crystalloids ( *center*) are present in tumor cells. By EM, these crystals are diagnostic (*inset*). Evidence favors a myogenous origin of this tumor, although myogenous differentiation has never been identified using EM (H&E, ×250). **G:** Leiomyosarcoma. Smooth muscle tumors in children are rare but do occur. Leiomyosarcoma in children and adults is characterized by spindle cells that resemble fibroblasts but stain intensely with eosin because of their content of smooth muscle actin-myosin bundles. Some degree of pleomorphism (*bottom center*) and mitosis (*upper center*) are often found (H&E, ×250). **H:** Liposarcoma. All liposarcomas are rare in children, and of the four types commonly seen in adults, only the myxoid type is common in children. This relatively low-grade sarcoma resembles fetal lipoblasts, often interspersed among more differentiated adipocytes (large clear cells). The vasculature ( *left*) is routinely prominent in liposarcomas and helps to differentiate this tumor from lipoblastoma and related benign fatty tumors (H&E, ×400).

| Tumor | MIC2 | Keratin | Epithelial membrane antigen | S100 | Neurofilament/ tubular protein | Vimentin |
|---|---|---|---|---|---|---|
| Fibrosarcoma | - | - | - | - | - | + |
| Synoviosarcoma | - | + | + | - | - | ± |
| Malignant peripheral nerve sheath tumor | - | ± | ± | + | ± | + |
| Ewing's sarcoma/primitive neuroectodermal tumor | + | ± | ± | - | - | ± |
| Desmoplastic small round cell tumor | - | ± | ± | - | - | + |

**TABLE 34-10. THE IMMUNOHISTOCHEMICAL PROFILE OF NONRHABDOMYOSARCOMA SOFT TISSUE SARCOMA**

### Clinical Presentation

Fibrosarcomas occur most frequently on the extremity, often in the distal segments; 70% of the reported cases of congenital fibrosarcoma occur at this site. [40],[41] and [42] In a review of 182 children with fibrosarcoma, the extent of disease was defined: 80% had localized disease, 8% had regional dissemination, and 12% had widespread disseminated disease.[187]

### Principles and Recommendations for Treatment

The overall approach to treatment of the older child with fibrosarcoma is different from the approach to an infant with congenital fibrosarcoma but similar to that for other NRSTSs. In contrast to congenital fibrosarcoma, the 5-year survival rate of fibrosarcoma in older patients is approximately 60%. [5] The most common site of metastasis in these older patients is the lung. The treatment principles are those outlined in the general discussion of treatment of NRSTS in children.

## MALIGNANT PERIPHERAL NERVE SHEATH TUMOR

### Epidemiology, Genetics, and Biology

MPNSTs account for approximately 5% to 10% of all NRSTSs in children.[29,30,31 and 32] MPNSTs occur in association with NF1, which is characterized by café au lait spots, axillary freckling, neurofibromas, skeletal dysplasia, learning disabilities, and a variety of neoplasms.[27] Approximately 5% to 16% of patients with NF1 develop MPNST. NF1 is a common autosomal dominant disorder that has been mapped to 17q11.2.[27] The gene has been cloned. Although its function remains unknown, the NF1 gene probably acts as a recessive tumor suppressor gene. An evaluation of two malignant tumors from NF1 patients revealed allele loss at informative loci on both the long and short arms of chromosome 17.[27]

In addition, reports of somatic deletion of the NF1 gene in a neurofibrosarcoma further suggest the hypothesis that the lack of a tumor suppressor gene is important in the pathogenesis of this tumor. Another study of ten patients with MPNST, of whom nine had NF1, found recurrent abnormalities of chromosomes 1, 11, 12, 14, 17, and 22. These data suggest that inactivation of the NF1 gene and loss of tumor suppression genes on 17p and 22q may be associated with neoplastic transformation. A point mutation at the p53 locus at 17p13 has also been demonstrated in an MPNST, further supporting a role for the p53 gene or other genes on the short arm of chromosome 17 in some of the malignancies seen in conjunction with NF1.[27] Additional reports have demonstrated multiple cytogenetic aberrations characterized by loss of chromosomes and multiple structural anomalies of diverse types.[188,189]

The increased incidence and earlier occurrence of neurofibrosarcomas in children and young adults with NF1 provide strong clinical evidence for the hypothesis that these tumors are caused in part by the LOH of a tumor suppressor gene.

### Pathology

The variety of histologic appearances of this tumor is rivaled only by the many variants of MFH in adult soft tissue sarcomas.[190,191,192,193,194,195,196,197 and 198] No one description does justice to this entity, in which in addition to expected fibrosarcoma-like elements, a wide variety of other histologies can be found, notably epithelial, glandular, and even cartilaginous or other heterotopic elements. No other pediatric soft tissue tumor is as variable in its appearance, raising questions as to whether it is a single entity. Nonetheless, current thinking groups all of these disparate tumors as one: MPNST.

Although superficially similar in appearance, MPNST must be differentiated from fibrosarcoma. The cells are usually more variable in size and shape, a herringbone pattern is usually absent, and often typical features of adult MPNST can be found in some areas of the tumor: a myxoid stroma, palisading of nuclei, and occasionally well-defined organoid arrays of nuclei (so-called Verocay bodies).[199] These features, common in benign schwannomas, are far less conspicuous in the malignant counterpart but can be diagnostic if present. Electron microscopy is by far the most useful method of establishing a specific diagnosis; Schwann cell differentiation, lacking only neurites within cytoplasmic concavities (mesaxons), is usually conspicuous ( Fig. 34-4B). Alternatively, immunocytochemistry with S-100 antibodies is often useful[200] (Table 34-10). Some tumors are exceedingly complex and contain additional elements—for example, muscle elements in malignant triton tumor, glandular elements resembling synovial sarcoma, cartilage, bone, and melanocytic elements (pigmented malignant nerve sheath tumor, clear cell sarcoma, melanoma of soft parts)—yet are, broadly speaking, still within this general category of MPNST.

MPNST may be unique among childhood sarcomas. First, it is well known that neurofibromatosis patients are at increased risk for development of MPNST. Second, the gene for NF1 has been cloned.[201,202] Finally, it has been shown that NF1 is deleted in cases of neurofibrosarcoma.[28] These initial observations suggest that NF1 abnormalities may be relevant to the genesis of MPNST in non-NF1 patients as well. They have already been identified in other neural tumors in children and adults.[203]

### Clinical Presentation

In a report of 24 children with MPNST, 16 had the associated neurofibromatosis syndrome. The most common primary sites were extremity (42%), retroperitoneum (25%), and trunk (21%); three tumors occurred in other sites. A second report of 25 patients had similar findings, with six patients having stage I tumors, five stage II, ten stage III, and four stage IV.[204] Of the 49 patients, only six (12%) had metastases at diagnosis.

### Principles and Recommendations for Treatment

Surgery plays a key role in the management of MPNST in children. Of the 24 patients in the first series described in the previous paragraph, 12 underwent gross total removal of the tumor, and nine of these were tumor-free survivors at 3 years.[204] In contrast, none of the 12 patients who could not have their tumors grossly removed were alive without disease at 3 years. In the second series of children, 8 of 11 patients with gross total resections were free of disease. In contrast, of the 14 patients with incomplete removal of tumor, only five achieved complete remission.[205] In these two small groups of patients, it appears that patients treated by complete removal with adequate margins and those treated by gross resection and irradiation to microscopic residual disease had similar and good outcomes. Irradiation therefore contributes to local control of MPNST in patients with microscopic disease after initial surgery.[204,205] This result is consistent with the experience in adult patients. As with other NRSTSs, the role of adjuvant chemotherapy is not established for MPNSTs.

Although chemotherapy can produce tumor regression in patients with unresectable and metastatic disease, no chemotherapy regimen has emerged that can produce an adequate disease-free survival rate in patients with advanced disease. The most commonly used regimens include vincristine, cyclophosphamide, dactinomycin, and doxorubicin.[204] None of the patients with gross residual disease after surgery in the series reported survived disease-free, despite the use of this chemotherapy in 10 of the 12 cases.[204] Although the optimal regimen has not been defined, the combination of ifosfamide and etoposide, a regimen highly active in the treatment of recurrent small round cell tumors of neural origin, produced partial or complete tumor regression in seven of the eight patients in a small series of patients with recurrent MPNSTs.[156] Treatment with the combination of ifosfamide and etoposide is now being explored in this tumor. For patients with advanced disease, the combinations of ifosfamide with Adriamycin and ifosfamide with etoposide are the most promising.

## MALIGNANT FIBROUS HISTIOCYTOMA

### Epidemiology, Biology, and Genetics

Although MFH was the most common histologic diagnosis in the NCI series of adult patients with extremity sarcomas (accounting for 25% of the series, or 53 of 211 patients), it is much less common in children.[6,65,206] In the St. Jude series in children, 5 (8%) of the 62 cases of NRSTS were diagnosed as MFH.[207] In the Societe Internationaled'Oncology Pediatrique (SIOP) mesenchymal malignancy trials (MMT84 and MMT89), MFH was the second most common NRSTS, accounting for 10% of the cases.[208] MFH rarely occurs in the neonatal period or during the first year of life.[209,210]

Cytogenetic analysis of short-term cultures has revealed chromosomal abnormalities in MFH ( Table 34-2).[211] It has been observed that tumors with 19p+ have a pronounced tendency to recur both locally and systemically.[211] Ring chromosomes have also been noted in MFH tumor cells.[212]

### Pathology

Stout[213] popularized the notion that many soft tissue sarcomas in adults were not simply fibrous lesions but rather possessed more than one tissue element (e.g., fat, fibrous tissue, tissue macrophages). This concept has been widely adopted, and the diagnosis of MFH is now more common than in the past. Not surprisingly, MFH is now also more commonly diagnosed in children as well.[214,215] The typical microscopic appearance resembles fibrosarcoma but is distinct therefrom by the absence of a herringbone pattern, the presence of marked cellular pleomorphism, the presence of multiple cell types (especially lipid-laden tumor cells), and an overall more malignant appearance (Fig. 34-4C). A storiform pattern of tumor cells (described as radiating fascicles of tumor cells at right angles to one another) is virtually diagnostic of the tumor.[216,217]

Two caveats regarding MFH in children must be noted. First, fibrosarcoma and even recurrent aggressive fibromatoses may develop the appearance of MFH on

recurrence.[167] Whether this represents bona fide MFH is unclear, but the association with an aggressive clinical course is clear. Second, angiomatoid MFH, the only common form of recognized MFH occurring as a primary tumor in young children (especially in those younger than 15 years), may not be true MFH after all. [218] Work on a large series of cases of angiomatoid MFH has documented numerous differences in phenotypic expression by immunocytochemistry, including the expression of myogenic markers.[218,219] This, combined with the distinctive histology (Fig. 34-4C) and unusually favorable prognosis, strongly suggests that this lesion is a unique soft tissue lesion in children and young adults and should be differentiated from the adult-type MFH. Grossly, this tumor is distinctly nodular. Although a prominent vascular pattern is often present within the central nodular region of these tumors, there is a disagreement as to whether this tumor can express myoid or vascular markers.[220,221] Overall, the tumor often appears myofibroblastic and benign. It is far less malignant in behavior as well. An estimated 1% of these tumors develops metastases, less than any other form of so-called MFH. Angiomatoid MFH still possesses metastatic potential in rare circumstances, however.

### Clinical Presentation

In a series of ten children with MFH aged 2 to 18 years, two tumors occurred on the trunk, three in the lower limbs, and one each in upper limb, scalp, and kidney [220]; two additional cases occurred near the orbit in a field irradiated for retinoblastoma. [220] MFH is one of the most common radiation-induced sarcomas, and this may provide a clue to its pathogenesis.[34] The most common site of metastasis is the lung, although metastases to the brain and other sites are also seen. As previously noted, the pulmonary metastases and local recurrences of infantile fibrosarcoma have been reported in some cases to have the pathologic pattern of MFH. [167]

### Principles and Recommendations for Treatment

Because of the rarity of this tumor in childhood, the approach to treatment of MFH is based primarily on the experience with adults. In a recent series of 107 adult patients with MFH, the 3-year survival rate was 72%. Patients with tumors located in the extremities had a better 3-year survival rate than those with tumors of the trunk or head and neck (81% vs. 54%, respectively).[221] The accepted initial management is wide local excision of the tumor. If the tumor arises in the extremity, limb-sparing operations with irradiation to the tumor bed have been as successful as amputations. The tumor must be small and appropriately placed to allow such an approach, however. Although some experience in adults suggests that adjuvant chemotherapy is of benefit, the role of adjuvant chemotherapy has not yet been established in children with this tumor.[61] In data from two small reports, wide local excision was associated with long-term survival in all 14 patients. [220,221] and [222] Onlya minority received chemotherapy. Thus, the role of extirpative surgery is the accepted approach to MFH in children; however, the role of adjuvant chemotherapy is not established.

MFH is a chemoresponsive tumor. In an adult series, 31% of the 38 patients evaluated responded to preoperative chemotherapy and radiotherapy. Chemotherapy with vincristine, dactinomycin, and cyclophosphamide with or without doxorubicin has produced objective tumor regressions in children with advanced disease; however, the optimal chemotherapy regimen for children with advanced disease has not yet been determined. [223,224,225,226,227] and [228] Four of five children treated by Raney and coworkers with group III or IV disease had complete (three patients) or partial (one patient) tumor regressions, and two remained disease-free at 4.6 and 5.4 years, respectively.[223] Responses to the combination of ifosfamide and etoposide have also been seen. [156] As with other children with advanced NRSTS, the combination of ifosfamide and Adriamycin is promising and requires evaluation in children.

The approach to treatment of angiomatoid MFH is different from that for classic adult-type MFH. Because the risk of metastasis is very low, adjuvant chemotherapy is not indicated. This tumor does occasionally metastasize, however. A recent report of a single patient with metastatic disease suggests that this lesion may be chemoresponsive, as well.[228]

## SYNOVIAL SARCOMA

### Epidemiology, Biology, and Genetics

Synovial sarcoma is one of the most common soft tissue tumors in adults.[64] In the AFIP series of 345 cases, the median age was 26.5 years; 72% of patients were younger than 40 years.[229] In adolescents and young adults, synovial sarcoma is the most common NRSTS.[6,229] In a series of 154 patients with NRSTS, synovial sarcoma accounted for 23% of the cases seen at St. Jude Hospital over a 29-year period.[230] In a POG prospective trial of adjuvant chemotherapy, synovial sarcoma accounted for 26% of the cases,[61] and in the SIOP mesenchymal malignancy trials (MMT84 and MMT89), synovial sarcoma accounted for 17% of the tumors.[207,208]

Synovial sarcoma is characterized cytogenetically by the translocation t(X;18)(q11;Xp11), which results in the fusion of the *SYT* gene located on chromosome 18q11 and one of three closely related genes *SSX1*, *SSX2*, or *SSX4* located on the Xp11 breakpoint.[14,15] and [16,231,232] The fusion of these genes creates a chimeric fusion transcript *SYT-SSX,* which results in an altered transcription pattern through SSX-mediated binding sites. The SYT-SSX1 and SYT-SSX2 transcripts can readily be detected using reverse transcriptase-polymerase chain reaction. In one study, the SYT-SSX1 transcript was detected in 42 of 77 cases (54%) of synovial sarcoma and the SYT/SSX2 in the remaining cases.[14,16] Biphasic synovial sarcomas are almost exclusively associated with SYT/SSX1 transcript. For patients with localized disease, the SYT/SSX2 predicted improved overall metastases-free survival and survival. [233] A strong association between SYT-SSX1 fusion transcript and a high (greater than 10%) Ki-67 index was noted in one series.[16]

### Pathology

This peculiar soft tissue sarcoma of older children and young adults is unique for its propensity to differentiate into two distinct elements: a spindle cell fibrous stroma virtually indistinguishable from fibrosarcoma and a distinct glandular component with absolute epithelial differentiation (Fig. 34-4D). No other sarcoma in children does this, with the exception of rare nerve sheath tumors also containing glands. Epithelial differentiation can also be encountered in epithelioid sarcoma, a superficial soft tissue sarcoma with a granulomatous appearance.[234] Mesothelioma is typically recognized for a similar biphasic appearance, but it is rarely seen in children. [235]

The usual tumor is biphasic; it is this unique characteristic that sets it apart from other soft tissue sarcomas, especially fibrosarcoma. The absence of the glandular epithelial component renders the diagnosis extremely difficult unless immunocytochemistry with keratin antibodies is used. In that case, the spindle cells in synovial sarcoma are positive, unlike those in fibrosarcoma or any other soft tissue sarcoma except epithelioid sarcoma. [236] As noted above, the characteristic cytogenetic abnormality t(X;18) is present in the vast majority of both tumors, biphasic and monophasic. [15,16,231,232] and [233,237]

It is important to compare the histology of the monophasic or poorly differentiated variants of synovial sarcoma to fibrosarcoma, as this is a common differential diagnostic dilemma. Histologically, there may be great similarity on superficial inspection between the two; however, common features of synovial sarcoma such as “staghorn” vascularity, compared to the interlacing bundles of tumor cells giving a “herringbone” pattern in fibrosarcoma, are helpful diagnostically. Certainly, any evidence of divergent cellular differentiation strongly suggests that the tumor is more than a fibrosarcoma and warrants serious consideration of synovial sarcoma. Ultimately, the distinction is far more reliably made using immunohistochemistry. The presence of keratins and epithelial membrane antigen in clusters, or even single cells, within the tumor virtually excludes fibrosarcoma.[238,239,240,241] and [242] The most reliable method of diagnosing monophasic synovial sarcoma is the use of cytogenetics as described above.[243]

### Clinical Presentation

The median age in most series of patients is in the third decade of life, with approximately 31% of cases occurring in patients younger than 20 years, with a median age of 13 years in one large series.[244,245,246,247,248,249] and [250] The male to female ratio is usually approximately 1.2:1.0 in most large series, suggesting a slight difference in incidence related to gender. The most common anatomic location in which the tumor arises is the lower extremity, often in the region of the thigh and knee. Seventy-four percent of the tumors presented in the lower extremity in one report and 60% in another. [244] The next most common site is the upper extremity. Approximately 15% to 20% occur on the head, neck, and trunk.[244,245,246,247,248,249] and [250] CTand MRI scans usually reveal a particular heterogeneous septate mass with calcification.[251,252] As with most NRSTSs, the most common site of metastatic disease is the lung, which comprised 94% of the sites of metastases in the AFIP series.[229] Unlike most other NRSTSs, synovial sarcoma more frequently spreads to regional lymph nodes. This occurred in 21% of the patients with metastases in the AFIP series; only 2 of 37 pediatric cases had lymphatic metastases, however.[229]

### Principles and Recommendations for Treatment

The factors reported to be of adverse prognostic significance are the presence of metastases, large tumor size (diameter greater than 5 cm), tumor invasiveness, grade 3 histology, older age, tumor with poor histologic differentiation, and bone or neurovascular invasion. [249,250,253,254] and [255] The disease-free survival for adult patients with localized tumors of the extremity has been reported to be approximately 70% in a prospectively treated group. [64,65] Eighty percent of the patients treated at St. Jude over a 30-year period with group I or II disease were alive at 5 years, compared with only 17% of the patients with group III or IV disease. [253] Of 16 children with synovial sarcoma treated on the SIOP MMT84 trial, 68% were alive and disease-free in long-term follow-up. [206] For 26 patients enrolled in the Cooperative Soft Tissue Sarcoma Study of the German Society of Pediatric Oncology, the 5-year event-free survival estimate was 72% when a multimodality treatment approach that incorporated ifosfamide, doxorubicin, and irradiation was used. [256] These results are similar to those reported by the same group on a previous trial in which 31 patients with synovial sarcoma had an overall event-free survival rate of 74% at 5 years using multi-agent chemotherapy and irradiation after initial tumor excision or biopsy.[244]

Because this tumor is relatively rare in children, the guidelines for its optimal treatment have not yet been established. Wide local excision is the treatment of choice to control the primary tumor. Analysis of the adult series suggests that limb-sparing procedures with limited surgical margins followed by RT to the tumor bed produce an overall outcome that is similar to the outcome of patients treated with amputation. [64] Because most children are growing rapidly, the optimal approach to these tumors in young patients is usually surgery alone, to avoid the effects of ionizing irradiation of growing bones and soft tissues. RT is often required to control microscopic disease, however. The effectiveness of irradiation in the permanent control of large-bulk disease has not been established.

Although synovial sarcoma is a chemoresponsive tumor, the role of adjuvant chemotherapy has not been established. Although the results of a subsequent German study using multi-agent chemotherapy and irradiation appear superior to those in the earlier German experience using radical surgery alone, data from a randomized trial have not confirmed this apparent benefit. [257] Tumor regressionsin patients with advanced disease have been documented with a number of chemotherapy regimens.[258,259,260] and [261] Furthermore, synovial sarcoma is among the most chemosensitive NRSTSs and often responds to ifosfamide- or doxorubicin-based regimens.[145,261,262,263,264,265] and [266] Of 14 patients with nonmetastatic synovial sarcoma treated with preoperative chemotherapy consisting of high-dose cisplatin and doxorubicin or high-dose ifosfamide (14 g per $m^2$) plus cisplatin and doxorubicin, 13 of 14 patients remained disease-free at a median follow-up of 37 months (range, 6 to 85 months).[267] Objective tumor regressions have also been seen with the combination conventional regimen of ifosfamide and etoposide. [156] Because the lungs are often the only site of metastatic disease, aggressive resection of pulmonary lesions has been of benefit. [64,268] The role of adjuvant RT in completely resected lung lesions remains controversial.

## HEMANGIOPERICYTOMA

### Epidemiology, Biology, and Genetics

Hemangiopericytoma is rare in children and accounts for approximately 3% of all soft tissue sarcomas in this age group. [6,7,8] and [9,160] In the St. Jude series of NRSTS, only 5 of the 62 patients had hemangiopericytoma.[6] Cytogenetic abnormalities have been found in some hemangiopericytomas. The simple translocations t(12;19)(q13;q13.3) and t(13;22)(q22;q11) have been noted, as have complex, multiple chromosomal abnormalities. [269,270] These tumors occur most commonly in infants and typically arise in the subcutis and oral cavity. Although these tumors often exhibit histologic features (e.g., increased mitotic activity and necrosis) that would indicate a poor prognosis in adults, the prognosis for infants with this tumor is usually excellent. [271,272] and [273] Aggressive metastatic and multifocal lesions do occur in infants, however. Hemangiopericytomas in older children are usually more aggressive and carry a higher incidence of metastatic disease. [272,273]

### Pathology

This soft tissue neoplasm, also first described by Stout, [213] can be benign or malignant. It was thought to be derived from vascular pericytes, the first layer of support cells adjacent to endothelial cells in normal vessels, but biologic evidence of this origin in most cases has not been forthcoming. A more likely explanation is that individual cells of this mesenchymally derived neoplasm may occasionally exhibit pericytic differentiation. Whether such differentiation is unique to this tumor remains to be seen, especially because hemangiopericytic pattern may be observed in many spindle cell tumors.

The ultrastructural appearance of these tumors occasionally supports pericytic differentiation. [274] Such cells can be detected in putative cases of hemangiopericytoma (Fig. 34-4E); however, this is the exception, not the rule. Rather, it is the tissue pattern of a uniform tumor with a staghorn vascular pattern and reticulin positivity around each tumor cell that characterizes the diagnosis. Whether these tumors are truly pericytic is open to question.

### Clinical Presentation

The most common primary site of disease is an extremity, especially the lower extremity. The retroperitoneum is the second most common site of disease, followed by the head and neck region and then the trunk. In infants, these tumors frequently arise in the tongue and sublingual region. The most common sites of secondary disease are the lungs and bone. Patients with hemangiopericytoma may present with hypoglycemia or hypophosphatemic rickets that resolve with removal of the tumor.[49,50]

### Principles and Recommendations for Treatment

The behavior of hemangiopericytoma in older children is similar to that in adult patients. [275,276,277] and [278] The overall 5-year survival rate in most adult series varies from 30% to 70%.[275,276,277] and [278] The most widely accepted therapeutic approach is wide local excision. [275,276] and [277] As with other soft tissue sarcomas, RT is used if complete surgical removal of the tumor cannot be accomplished.[64] Local control has been achieved in this fashion with both microscopic and macroscopic disease remaining after surgery.[279] Three reports have documented the benefit of radiation in local control of these tumors even if there is gross residual disease remaining after chemotherapy.[280,281] and [282] In one small series, all four patients initially treated with surgery and postoperative RT remained alive with no evidence of disease even though three had gross residual disease at the time of irradiation. [283] The role of adjuvant chemotherapy has not been established; however, the high incidence of metastatic disease and the relative chemoresponsiveness of this tumor have led many investigators to treat these patients with adjuvant chemotherapy after extirpation of the primary tumor. Responses to chemotherapy with advanced disease have been reported with the use of vincristine, cyclophosphamide, doxorubicin, dactinomycin, methotrexate, mitoxantrone, and other alkylating agents.[47,284]

Hemangiopericytoma in infants, although similar in histologic appearance to the adult form, usually follows a more benign course. [45] The treatment of choice for infantile hemangiopericytoma is surgery alone if the tumor is localized.

## ALVEOLAR SOFT PART SARCOMA

### Epidemiology, Biology, and Genetics

Alveolar soft part sarcoma (ASPS) is a rare sarcoma that usually arises in persons aged between 15 and 35 years. [285,286] and [287] In most adult series, it accounts for less than 1% of the cases.[64,288] Although 6 of the 62 patients in the St. Jude series had ASPS,[6] the actual incidence in children and adolescents is probably lower. [7,8] and [9] In the SIOP mesenchymal malignancy trials, 5 of 122 children had ASPS.[207]

The prognosis of this tumor is related to the presence of metastases, the extent of local disease, and the location of the tumor. Primary sites common in children, the orbit and the head and neck, are associated with a more favorable outcome compared with ASPS in adults. Chromosomal analysis of several cases has shown an abnormality at 17q25; the ASPL-TFE3 gene fusion has recently been cloned. [289,290]

### Pathology

ASPS is of uncertain histogenesis.[291] By far the most distinctive feature of this sarcoma is the presence of periodic acid–Schiff–positive, diastase-resistant inclusions in the cytoplasm by light microscopy (Fig. 34-4F). By electron microscopy, they show a regular crystalline structure (Fig. 34-4F, inset). Their biochemical nature is

unknown, although adenosine triphosphatase has been found in the tumors by some, suggesting myogenic differentiation. [292] No biologically active secretory product has been detected in a tumor or patient. The finding that some inclusions closely resemble neurosecretory granules has provoked suspicion that the tumor may be neuroepithelial, although other researchers have found seemingly identical inclusions in muscle. [292,293] Immunocytochemical data have overwhelmingly supported a myogenic phenotype for this tumor despite the lack of any known normal tissue counterpart. [294,295,296,297,298,299,300,301 and 302] Nonetheless, in light of its unusual clinical behavior, this tumor should not be grouped with alveolar rhabdomyosarcoma clinically or therapeutically.

### Clinical Presentation

ASPS usually presents as a slow-growing, asymptomatic mass. The tumor usually occurs in the skeletal muscle of the extremities [285,286,287 and 288]; however, in children, the head and neck region is a common site. The most frequent sites of occurrences in the head and neck are orbit and tongue. [303] The clinical course of ASPS is often indolent. Despite the fact that more than 80% of children and young adults with ASPS are alive 2 years after diagnosis, most patients die of this disease, sometimes as long as 20 years after the diagnosis is made. [304,305] The most common metastatic site is the lung, followed by brain, bone, and lymph node. [304,305]

### Principles and Recommendations for Treatment

Because of the indolent clinical course, the initial therapeutic approach is usually complete local excision alone, with irradiation and chemotherapy reserved for treatment of recurrent disease. [305,306 and 307] With this standard approach, however, most patients eventually relapse and subsequently die of disease. This ominous fact strongly suggests that new approaches to the prevention of relapses are needed to treat this disorder. Younger patients may have a better prognosis; however, younger age is associated with more favorable primary sites. Improved disease-free intervals exist for orbital ASPS compared to nonorbital ASPS, and for head and neck ASPS compared to extremity ASPS. Aggressive attempts to surgically remove metastatic disease should be made in light of the indolent course. This may be especially true for brain metastases. [308] RT may be beneficial for patients when complete extirpation of the tumor is not achievable. All 12 patients with metastatic disease in one series had meaningful palliation of extraskeletal disease with radiotherapy; six patients without metastatic disease but with large local tumors had prolonged local control with RT. [309] Responses to chemotherapy have been reported, primarily with anthracycline-based regimens. In a more recent report of the St. Jude series, 9 of 11 patients survived long term even though chemotherapy did not produce responses. [309a]

## LEIOMYOSARCOMA

### Epidemiology, Biology, and Genetics

Although leiomyosarcomas account for approximately 7% of all soft tissue sarcomas in adults, this tumor is rare in childhood, accounting for fewer than 2% of cases. [310,311 and 312] Only three leiomyosarcomas were reported in a large series of 135 soft tissue tumors in infants and children from the Mayo Clinic. [313]

The role of irradiation as a predisposing factor for leiomyosarcoma is disputed; however, the occurrence of leiomyosarcoma in skin, subcutaneous tissue, and other soft tissues after irradiation has been reported. [314,315,316,317,318 and 319] They are much less common as secondary tumors than MFH, fibrosarcoma, or osteosarcoma. Leiomyosarcomas have also been reported in individuals with bilateral retinoblastoma, [314,315] occurring both within and outside the radiation field. This suggests that the genetic abnormality at the RB1 locus may be important in the pathogenesis in this tumor. Adding more evidence to support this hypothesis is a recent report that mutations at the RB1 locus occur in some sporadic leiomyosarcomas. [320]

Although smooth muscle tumors have been rare in children, the appearance of HIV infection in adult and pediatric populations has altered the normal incidence of both smooth muscle and vascular tumors in both age groups in some populations. [35,36 and 37] Previously unreported tumors with such differentiation are increasingly being reported, sometimes multifocal and with varying phenotypes, including apparently benign and malignant smooth muscle tumors. Of interest is the report of EBV linked to leiomyomas and leiomyosarcomas in children with acquired immunodeficiency syndrome. [37] This is the first example of EBV associated with a soft tissue sarcoma, although it is well known to be associated with Hodgkin's disease and lymphoproliferative syndromes. There are now 12 such cases of leiomyosarcoma in patients who are HIV-positive. EBV genomes have been identified in the leiomyoma and leiomyosarcoma cells of HIV-infected patients. These tumors have also been reported after treatment for acute lymphocytic leukemia [38] and during immunosuppression to prevent renal allograft rejection. [39] In addition, leiomyosarcomas have been reported in patients with Hodgkin's disease in irradiated fields and in the urinary bladder after cyclophosphamide treatment. [321] These reports raise the question of whether the immune defect associated with the underlying disease somehow interacts with the therapy to predispose the tissue to development of this tumor. A t(12;14) translocation has been reported in a leiomyosarcoma arising in a child. The same translocation has been found in leiomyosarcomas arising in adults and in uterine leiomyosarcomas. This suggests that genes arising near the breakpoint of t(12;14)(q 14-15;q23-24) are probably important in the pathogenesis of benign and malignant smooth muscle tumors. [322,323] Other authors have suggested three patterns of cytogenetic abnormalities: (a) hypodiploid; (b) pseudodiploid, associated with reciprocal translocations; and (c) heterogeneous karyotypic findings. [324]

### Pathology

Leiomyosarcoma is normally an exceedingly uncommon soft tissue sarcoma in children but has been documented in one large series only recently. [325,326] Most smooth muscle–appearing tumors are in fact variants of embryonal rhabdomyosarcoma (type A, or leiomyomatous rhabdomyosarcoma) and carry an excellent prognosis. [327] Nonetheless, occasional tumors with bona fide smooth muscle differentiation, indistinguishable from the adult counterpart, are encountered. The tumor cells are elongated, with cigar-shaped nuclei and brightly eosinophilic cytoplasm (because of the content of myofilaments); they are closely packed in parallel arrays ( Fig. 34-4G). The appearance is superficially similar to fibrosarcoma, but the eosinophilic cytoplasm, the nuclei resembling smooth muscle in normal tissues, and the usual monotonous regularity of tumor cells are clearly distinct therefrom. Rarely, tumors with combined phenotypic characteristics, such as adipocyte and smooth muscle differentiation, have been reported. [328]

### Clinical Presentation

The most common primary sites in adults are the retroperitoneum, peripheral soft tissue, and gastrointestinal tract. [310,311,312 and 313,329,330 and 331] Otherless common sites are vascular sites and the subcutaneous region. In contrast, the most common primary site in children is the gastrointestinal tract, especially the stomach. [329] Leiomyosarcoma arises less commonly in the retroperitoneum, in the peripheral soft tissue, and in the genitourinary tract. This tumor has also been reported to arise in the orbit, perineum, saphenous vein, bladder, cecum, colon, and ovary of children. It is clear that they may arise in almost any soft tissue or vascular structure. [332,333,334,335,336,337 and 338]

A particularly important clinical presentation to recognize is the gastric epithelioid leiomyosarcoma, which may be associated with extra-adrenal or adrenal paraganglioma and pulmonary chondroma: Carney's triad. [339,340] This syndrome may present with the full triad but commonly does not. It typically presents in younger individuals, more commonly young women, usually with symptoms related to the gastric tumor. [339,340] The patients must then be monitored closely for the occurrence of the other lesions. This gastric sarcoma may progress very slowly even with the presence of metastatic disease, unlike the conventional gastric leiomyosarcoma.

### Principles and Recommendations for Treatment

The most common approach to the treatment of these tumors has been wide local excision alone. [310,311 and 312,329,330 and 331] The roles of chemotherapy and RT in the treatment of leiomyosarcoma in children is not yet known. If complete extirpation of the tumor can be achieved, the prognosis is usually good for tumors arising outside the gastrointestinal tract; however, patients with tumors arising in the gastrointestinal site usually have a poor prognosis. [310,331,341] Leiomyosarcomas of the colorectal region in children, although extremely rare, appear to have a relatively good prognosis if the tumor can be completely excised. [330]

In children with advanced leiomyosarcoma, there is limited experience with chemotherapy. Responses have been seen with MAID, CYVADIC, and other combination chemotherapy regimens. [153,342,343] Adriamycin and DTIC appear to have the best activity against this disease. In contrast, ifosfamide with or without etoposide does not appear to have any significant activity against leiomyosarcoma. [156]

Epithelioid leiomyosarcoma of the stomach is a benign-acting lesion that must be differentiated from true leiomyosarcoma. Although this tumor may rarely

metastasize, it can almost always be cured with local excision. Long-term survival has been reported in the face of metastatic spread, further supporting the conclusion that this tumor has benign behavior. [344,345,346,347 and 348]

## LIPOSARCOMA

### Epidemiology, Biology, and Genetics

Liposarcoma is one of the most common soft tissue sarcomas in adults; it accounts for 5% to 18% of cases in most series. [64,349,350] Although it is primarily a disease of adults, with a peak age incidence of 40 to 60 years, this tumor may occur in children, most often in the early part of the second decade of life. [351,352 and 353] The tumor may rarely affect infants and young children, in whom its behavior is almost always benign if complete removal can be achieved. [351] As is the case with many of the sarcomas discussed in this chapter, recent laboratory studies have identified a recurrent genetic abnormality that is probably of both etiologic and diagnostic value. The consistent cytogenetic findings in myxoid liposarcoma of a t(12;16)(q13;p11) translocation strongly suggests that a gene in the region of 12q13 plays an important role in the pathogenesis of this tumor. [322,354,355,356 and 357] Specifically, fusion of a transcription factor (CHOP), essential for adipocytic differentiation to another gene (TLS or EWS), has been documented in myxoid liposarcoma. [17,18 and 19] This observation has been extended to round cell liposarcoma as well. [358] No such recurring molecular genetic abnormality has yet been described in the other two major forms of liposarcoma (i.e., well differentiated and pleomorphic), nor has the incidence of this abnormality within round cell and myxoid liposarcoma yet been documented. Lipomas also have structural changes in chromosome 12q13-q14, suggesting that the degree of abnormality at this locus may be related to the growth of lipogenic tumors. [322,355]

### Pathology

To many, liposarcoma is not a tumor of childhood. Most pathologists have been loath to diagnose malignancy in a soft tissue neoplasm with lipoblastic differentiation, preferring instead to diagnose lipoblastomatosis or lipoma. This prejudice is unwarranted in view of the documented examples of liposarcoma in children. [359,360,361 and 362] As in adults, the tumor may be well differentiated, myxoid, round cell, or pleomorphic, in increasing degrees of malignancy and with decreasing rates of survival. Myxoid liposarcoma is the most common histologic subtype in adults, accounting for 45% to 55% of all liposarcomas. [363] The myxoid subtype is also the most common in children: in one series, 13 of the 18 tumors were the myxoid subtype. [362] A typical liposarcoma myxoid type is illustrated in Figure 34-4H. Most tumor cells are fibroblastic; only rare cells show conspicuous lipoblastic differentiation. The distinction between liposarcoma and MFH can be difficult, but the presence of a myxoid stroma, conspicuous small blood vessels, and scant mitotic activity are all typical of liposarcoma, unlike MFH or even fibrosarcoma.

### Clinical Presentation

The two most common primary sites are the lower extremity and trunk. The most common lower extremity site is the thigh-knee region; the most common truncal site is the retroperitoneum. Although metastases are uncommon, the most frequent site is the lung in most series; however, a recent review of 60 adult patients with liposarcoma found that isolated extrapulmonary disease was the site of initial metastasis in 59% of patients with metastases. [364] This suggests that in comparison with other soft tissue sarcomas there is a higher tendency toward extrapulmonary sites. [364] In addition, these investigators observed that these patients in this small series had a longer disease-free interval compared with other patients with pulmonary disease.

### Principles and Recommendations for Treatment

Because these tumors rarely metastasize but can be locally invasive, the treatment of choice for localized liposarcoma is wide local excision alone. [351,352 and 353] For retroperitoneal tumors, this strategy may not be possible, however. Local recurrences may ultimately result in death of the patient by extension of the tumor into vital structures despite the absence of metastatic disease. A recent series of patients younger than 23 years demonstrated the importance of completely resecting this tumor. [362] Irradiation appears to be effective in the control of microscopic disease in adult series [64]; however, its role in the treatment of children has not yet been determined. Irradiation should be strongly considered for retroperitoneal liposarcomas in children.

The role of adjuvant chemotherapy in the treatment of liposarcomas of childhood has not yet been defined; nevertheless, the long-term prognosis for children with this rare tumor is very good with surgery alone as long as the tumor can be removed with adequate surgical margins. [351,352 and 353]

A small series of children with liposarcoma with microscopic residual disease treated with EBRT has been reported; two of three children remained disease-free at 2 and 11.8 years after diagnosis. [362] The effectiveness of irradiation and chemotherapy in the treatment of gross residual disease has also not yet been established, but there are reports of responsiveness to chemotherapy.

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# 35

# OSTEOSARCOMA

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## INTRODUCTION

In the 1920s, a celebrated surgeon of international repute summarized a scientific meeting on bone sarcomas by acknowledging, “If you do not operate, they die; if you do operate, they die just the same. Gentlemen, this meeting should be concluded with prayers.”[1] One might have drawn a similarly disheartening conclusion from meetings on osteosarcoma conducted in the 1960s and early 1970s: Few therapeutic advances had occurred during the preceding half century, and the outlook for patients with osteosarcoma was indeed dismal. Despite aggressive surgery, more than 80% of children presenting without evidence of metastases developed tumor recurrences and died of their disease within 5 years of diagnosis.

In the last three decades, remarkable progress has been made in our understanding of the biology and etiology of osteosarcoma and in the treatment of affected patients. As a result of the development of effective adjuvant therapy and advances in surgical and diagnostic imaging techniques, the majority of patients who have limb primary tumors and present without metastases can now be cured. Advances in surgical techniques also have permitted improvements in the quality of life of survivors through the use of limb-sparing surgical procedures to control the primary tumor. Explosive advances in genetics and molecular biology have provided new insights into mechanisms of tumorigenesis and have suggested novel avenues of research.

## EPIDEMIOLOGY

Osteosarcoma is a primary malignant tumor of bone, deriving from primitive bone-forming mesenchyme and characterized by the production of osteoid tissue or immature bone by the malignant proliferating spindle cell stroma.[2,3] and [4] Although rare in childhood (metastatic lesions to the skeleton are more common), primary bone tumors are the sixth most common group of malignant neoplasms in children[5]; in adolescents[6] and young adults, they are the third most frequent neoplasms, exceeded in older children only by leukemias and lymphomas. Malignant bone tumors have been reported to occur in the United States at an annual rate of approximately 8.7 cases per million children and adolescents younger than 20 years. [5] Only one-half the bone tumors in childhood are malignant[6]; of these, osteosarcoma is the most frequent,[2,6,7] accounting for 35% of all primary sarcomas of bone and 56% of the malignant bone tumors in the first two decades of life. [6] (Ewing'ssarcoma, the second most frequent primary bone cancer, actually is more common than osteosarcoma in children younger than 10 years.) Each year in the United States, osteosarcoma is diagnosed in approximately 400 children and adolescents younger than 20 years. [5] Most series show boys to be affected more frequently, and the incidence in black children is higher than that in whites. [2,5,8,9]

The peak incidence of osteosarcoma occurs in the second decade of life during the adolescent growth spurt ( Fig. 35-1), a feature that suggests a relationship between rapid bone growth and the development of this malignancy. Several lines of evidence have been cited to support this relationship. First, patients with osteosarcoma are taller than their like-aged peers. [10] Similarly, the risk of developing osteosarcoma in breeds of large dogs (e.g., the Great Dane and the St. Bernard) has been reported to be nearly 185 times that for small breeds. [11] Second, osteosarcomas occur at an earlier age in girls than in boys, corresponding to the more advanced skeletal age and earlier adolescent growth spurt of girls, [12] whereas the increased risk for osteosarcoma among boys may result from the larger volume of bone formed during a longer growth period. Third, osteosarcoma has a predilection for the metaphyseal portions of the most rapidly growing bones in adolescents (the distal femur, proximal tibia, and proximal humerus), and tumors of the humerus tend to occur at a younger age than do tumors of the femur and tibia, corresponding to the earlier growth spurt of the humerus. [12] Thus, the tumor appears to occur most frequently at sites where the greatest increase in length and size of bone occurs. This has led to the speculation that bone tumors arise from an aberration of the normal process of bone growth in length and remodeling [12,13]; rapidly proliferating cells might be particularly susceptible to oncogenic agents, mitotic errors, or other events leading to neoplastic transformation. [10]

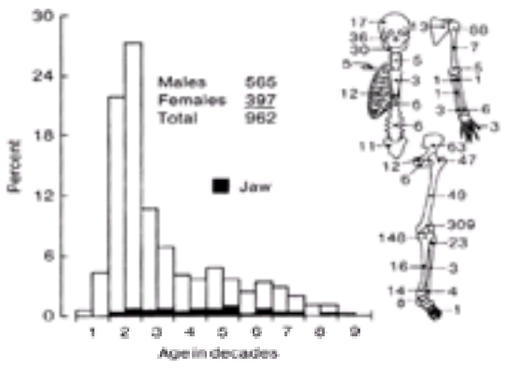



**FIGURE 35-1.** Age, gender, and skeletal site distribution of osteosarcomas in a large series of patients from the Mayo Clinic. (Reproduced with permission from DC Dahlin. Osteosarcoma of bone and a consideration of prognostic variables. Cancer Treat Rep 1978;62:189–192.)

## ETIOLOGY AND GENETICS

The etiology of osteosarcoma is unknown. A viral etiology was suggested by evidence that bone sarcomas can be induced in select animals by viruses [14,15] or cell-free extracts of human osteosarcoma.[16] Support for an association of an infectious agent with osteosarcoma also derives from studies demonstrating anti-sarcoma-specific antibodies in patients and in close relatives of patients with sarcomas. [17,18] and [19] In addition, lymphocytes cytotoxic to osteosarcoma cells have been found in the peripheral blood of both patients with osteosarcoma and their parents. [20] However, no convincing data have emerged from the laboratory to demonstrate a causative

infectious agent, and such speculation has been discarded. Antecedent trauma often has been associated with the development of bone tumors, but little evidence exists to support a causal relationship. Rather, injury (particularly pathologic fracture) often brings the patient to medical attention, and radiographs reveal the underlying neoplasm.

The only environmental agent known to produce bone sarcomas in human beings is ionizing radiation. Radiation is implicated in approximately 3% of osteosarcomas,[8,9] although the incidence of postirradiation sarcoma is low in view of the extent to which therapeutic irradiation has been used. [2,21] An increased incidence is likely to be seen as more patients survive long enough after primary irradiation to develop this complication. Osteosarcomas resulting from therapeutic irradiation initially were seen after high orthovoltage radiation doses to bone (see Chapter 13).[22] More recently, osteosarcomas have been reported also as a complication of megavoltage irradiation,[23] although the incidence after megavoltage irradiation may be lower because absorbed bone doses are reduced considerably with this technique.[23,24] The interval between irradiation and the appearance of osteosarcoma has ranged from 4 to more than 40 years (median, 12 to 16 years), [2,25] and bone sarcoma has occurred after irradiation for both benign and malignant conditions. Noteworthy is that osteosarcomas have been associated also with the use of bone-seeking radioisotopes,[26] such as intravenous radium 224 for the treatment of ankylosing spondylitis and tuberculosis, [26,27] and the use of Thorotrast as a diagnostic radiocontrast agent.[28]

Treatment with alkylating agents also may be linked to the subsequent development of bone cancer independent of the administration of radiotherapy. The risk of subsequent bone cancer appears to rise with increasing drug exposure, and the administration of alkylating agent chemotherapy may potentiate the effect of radiation in the development of secondary osteosarcomas.[29] Treatment with anthracyclines appears to shorten the interval to the development of secondary bone sarcomas. [30]

Osteosarcoma has been reported in patients with Paget disease, and cases of osteosarcoma in patients older than 40 years are associated almost exclusively with this premalignant condition.[2] Approximately 2% of patients with Paget disease develop osteosarcoma, and the occurrence of osteosarcoma is not necessarily related to the extent of involvement of the skeleton by Paget disease.[2] Histologically, osteosarcomas in patients with Paget disease are indistinguishable from conventional osteosarcoma, although multiple bone involvement is frequent, [2] and the prognosis for such patients is poor. [9] Other benign bone lesions also are associated with an increased risk of the development of osteosarcoma. Lesions predisposed to such malignant degeneration include solitary or multiple osteochondroma, solitary enchondroma or enchondromatosis (Ollier disease), multiple hereditary exostoses, fibrous dysplasia, chronic osteomyelitis, sites of bone infarcts, and sites of metallic implants for benign conditions.[2,9,31,32]

Several families have been described in which multiple members have developed osteosarcoma,[2,33,34] suggesting that a genetic predisposition to this tumor exists. By far the strongest genetic predisposition to osteosarcoma is found in patients with hereditary retinoblastoma (see Chapter 28). The subsequent development of second nonocular tumors in patients who survive retinoblastoma has been reported in many case reports and series. The majority of second malignancies are sarcomas, and almost 50% are osteosarcomas.[35,36] The actuarial risk for the development of a secondary tumor among patients with hereditary retinoblastoma has been estimated to be between 8% and 90% at 30 years.[37,38,39 and 40] Although the occurrence of secondary osteosarcoma in survivors of retinoblastoma was initially thought to represent a complication of radiotherapy,[35] it is now apparent that a relationship between osteosarcoma and hereditary (bilateral) retinoblastoma exists that is independent of therapy, since the secondary cancers virtually always occur in patients with hereditary retinoblastoma with germ line deletion [37] (although the sporadic form of retinoblastoma is more common), occur in sites far distant from irradiated tissue (e.g., in the extremities), and occur in patients who did not receive irradiation as part of therapy for retinoblastoma. In hereditary retinoblastoma, osteosarcomas occur 2,000 times more frequently in the skull after radiotherapy and 500 times more frequently in the extremities than would be expected in the general population. [37,41] The available evidence suggests that retinoblastoma is one of several childhood tumors that arise as a result of recessive mutations[41,42 and 43]; several studies confirm that the specific locus involved in the generation of retinoblastoma (mapped to band q14 of chromosome 13 and termed the *RB susceptibility locus*) is implicated in the generation of osteosarcoma as well,[44,45,46,47 and 48] even in patients without a prior history of retinoblastoma. Tumor cells from some osteosarcomas and osteosarcoma cell lines (from patients without retinoblastoma) were found to demonstrate specific loss of constitutional heterozygosity for loci scattered on chromosome 13 [46,47 and 48] or to demonstrate complete chromosome 13 homozygosity,[44,47] although analysis of constitutional (nontumor) DNA from the same patients indicated heterozygosity at these same loci. Further studies have found deletions or rearrangements of the *RB* gene, absence of RNA transcripts corresponding to the retinoblastoma locus, [45] or altered expression of the *RB* gene product in osteosarcomas and osteosarcoma cell lines.[46] The RB protein is a 105-kd nuclear phosphoprotein, which binds to DNA and is phosphorylated by cyclin-dependent kinases during the S and $G_2$/M phases of the cell cycle, but its exact function remains to be determined. The experimental introduction (by retrovirus-mediated gene transfer) of a cloned *RB* gene into osteosarcoma cells that lack the *RB* gene product results in restoration of the expression of the RB protein in such tumor cells and a change in cell morphology, significant inhibition of tumor cell growth in culture, inhibition of the formation of tumor colonies on soft agar, and suppression of tumor formation in nude mice.[49] Apparently, a common mechanism for tumorigenesis in retinoblastoma and some osteosarcomas involves the unmasking of recessive mutations at a locus exerting pleiotropic tissue effects.[47] Thus, defects in both retinoblastoma alleles occurring in an appropriate *bone* cell may lead to osteosarcoma much in the same way that defects in both alleles of the *RB* gene in a retinoblast leads to the generation of retinoblastoma. [44] Rapidly proliferating regions of bone may be particularly susceptible to mitotic errors, which might result in homozygosity of a defective retinoblastoma allele and tumor formation. Recent studies indicate that the initial mutations of the *RB* gene in sporadic osteosarcomas occur predominantly in the paternal gene,[50] suggesting the involvement of germinal imprinting in producing the differential susceptibility of the two genes to mutation.

Many osteosarcomas and osteosarcoma cell lines apparently express normal-sized RB protein and have normal RB alleles, implying that for some osteosarcomas, alternative pathways of tumorigenesis exist independent of *RB* gene inactivation.[49] Further investigations have implicated a second recessive oncogene, p53, in the etiology or progression of osteosarcoma. The gene, which has been mapped to the chromosome region 17p13.1, encodes a 53-kd nuclear phosphoprotein that binds DNA and is believed to be involved in regulation of DNA replication and, thus, to be involved in control of the cell cycle. [51] The p53 gene product appears to be critical in maintaining the integrity of the genome. In normal cells, the presence of DNA damage results in accumulation of p53, which switches off replication to allow time for DNA repair. If repair of DNA damage is unsuccessful, p53 may trigger apoptosis, thus inducing cellular suicide. Cells with mutant or inactivated p53 cannot respond appropriately to DNA-damaging agents and accumulate mutations at an increased rate, leading to malignant transformation. [52]

Several lines of evidence implicate an important role for p53 in malignant transformation in osteosarcoma. First, the chromosome region 17p12-17p13.3 has shown frequent losses of heterozygosity in a variety of tumors, notably osteosarcoma,[53] and this region is a frequent locus of karyotypic change (particularly deletion of the short arm of chromosome 17) in osteosarcomas. Further, transgenic mice containing a highly expressed mutated p53 gene [54] and transgenic mice with both p53 alleles destroyed by an insertion mutation [55] have an increased incidence of many cancers, particularly osteosarcomas. Studies of osteosarcomas and osteosarcoma cell lines have demonstrated a substantial proportion that exhibit detectable alterations in p53, including homozygous deletions of the p53 locus, loss of heterozygosity at loci flanking p53, or failure of expression of p53 protein or expression of p53 proteins of aberrant size. [51,56,57,58 and 59] Mutations of the p53 gene are detectable in almost 25% of cases of osteosarcoma,[60] and such mutations prove to be fairly heterogeneous in their distribution in the gene and the type of genetic alteration that results. Inactivation of the p53 gene product appears to be important for the growth of many osteosarcomas, presumably as a result of loss of a growth regulator that normally constrains cellular proliferation. [51,57] Moreover, transfection of single copies of wild-type p53 are sufficient to suppress the neoplastic phenotype of human osteosarcoma cells lacking p53 expression and of cells expressing a mutated p53 allele. [61]

The p53 gene product normally interacts with other cellular proteins so that mutations in genes coding for proteins required for p53 functions may affect p53 activity indirectly and thus facilitate malignant transformation. A cellular phosphoprotein, MDM2—the product of the murine double minute 2 gene that binds to both wild-type and mutant forms of the p53 gene product—appears to be involved in transcriptional regulation and may regulate some aspects of p53 activity [62] or may functionally inactivate p53.[63] The human MDM2 gene has been localized to chromosome 12q13-14,[63] a region that has been found to be amplified in approximately 20% of osteosarcoma tumor specimens.[64] Preliminary results indicate that in tumors demonstrating MDM2 amplification, no mutations in p53 are found. Thus, high levels of MDM2 may inactivate the tumor suppressor activity of p53 by complexing with it through mechanisms analogous to those employed by viral oncoproteins, such as SV40 T antigen and adenovirus E1B.[52,63] Amplification of MDM2 production thus may have the same functional effect as mutations of the p53 gene. Notably, in one study, amplification of MDM2 was detected in metastatic and recurrent osteosarcomas but not in primary tumor specimens,[65] suggesting that the MDM2 gene product may be involved in tumor progression rather than primary tumorigenesis. Likely, other partners of p53 capable of binding p53 may be important in tumorigenesis as well.[52]

In addition to its appearance in retinoblastoma kindreds, osteosarcoma occurs also as part of another familial cancer syndrome in which affected families are afflicted with a spectrum of cancers, including carcinoma of the breast, soft tissue sarcomas, brain tumors, leukemia, adrenal cortical carcinoma, and osteosarcoma. [66,67] Affected members of such families with the so-called Li-Fraumeni syndrome have a projected risk of developing invasive cancer of 50% by age 30 and more than 90% by age 70.[68] The p53 gene has been found to play a pivotal role in the Li-Fraumeni syndrome, because affected kindreds have been found to inherit germline mutations of this gene.[68] Screening of large series of children with osteosarcoma reveals that approximately 3% to 4% carry constitutional germline mutations in p53.

The majority of these cases with germline p53 mutations occurred in patients with a strong family history of cancer, with family histories suggestive of the Li-Fraumeni syndrome, or in patients with multiple cancers.[69,70] Thus, osteosarcoma may appear as the initial or subsequent malignant neoplasm in cancer-prone individuals, although likely such patients represent only a small proportion of children with osteosarcoma.

Two recessive oncogenes—p53 and RB—thus have major roles in tumorigenesis in osteosarcoma. Both RB and p53 are involved in cell cycle regulation, and both pathways are inactivated in the majority of human cancers. Other genes almost certainly are involved in these processes. Analysis of DNA from primary tumors and corresponding normal cells from patients with osteosarcoma demonstrates a high frequency of allele loss at loci on chromosomes 3q and 18q (in addition to 13q and 17p, the known loci of RB and p53, respectively), suggesting that at least two additional tumor suppressor genes are involved in the development of osteosarcoma.[71,72] and [73]

## PATHOLOGY

The diagnosis of osteosarcoma is based on histopathologic criteria and correlation with a confirmatory radiologic appearance. The histologic diagnosis of osteosarcoma depends on the presence of a frankly malignant sarcomatous stroma associated with the production of tumor osteoid and bone ( Fig. 35-2). Great variability exists in the histologic patterns seen in this tumor and in the degree of osteoid production, so that extensive review of the pathologic material and, rarely, electron microscopy may be required to demonstrate tumor osteoid.

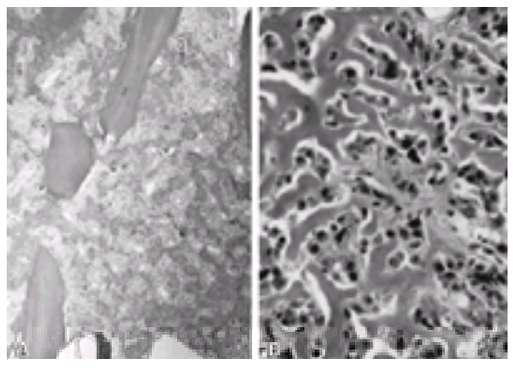
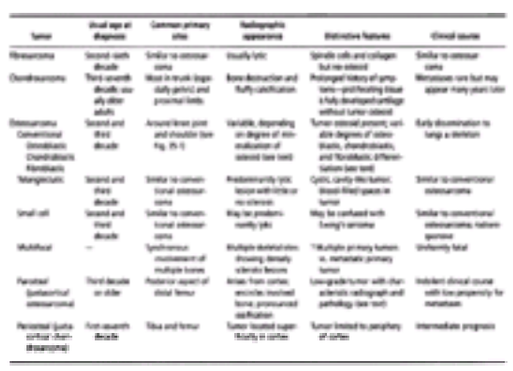


**FIGURE 35-2. A:** Photomicrograph of a field of an osteosarcoma demonstrating extensive osteoid formation intimately associated with a sarcomatous stroma (×100). **B:** High-power view demonstrating cellular atypia and other features of the malignant cellular stroma (×400).

Because osteosarcomas are thought to arise from a mesenchymal stem cell capable of differentiating toward fibrous tissue, cartilage, or bone, [74] osteosarcoma shares many features with chondrosarcoma and fibrosarcoma, tumors of the same family (generically termed *osteogenicsarcomas*) with which osteosarcoma is easily confused (see Chapter 8 and Chapter 38).[4] However, chondrosarcomas and fibrosarcomas are distinguished from osteosarcoma by their lack of production of osteoid substance, the *sine qua non* for the diagnosis of osteosarcoma. Features that distinguish chondrosarcoma and fibrosarcoma from osteosarcoma are summarized in Table 35-1. A number of distinct clinicopathologic variants of osteosarcoma have been defined based on clinical, roentgenographic, and histologic features ( Table 35-1). The largest group of osteosarcomas are the conventional osteosarcomas ( Fig. 35-2, Fig. 35-3), the variants of which are seen predominantly in children and adolescents. In conventional osteosarcoma, the connective tissue stroma variably appears as a mixture of large, atypical, spindle-shaped cells that are cytologically highly malignant, with large irregular nuclei and abnormal mitotic figures. The stroma may be largely anaplastic. Large numbers of benign-appearing giant cells may be evident in up to 25% of cases. Interspersed in the pleomorphic stroma are areas of osteoid production and calcification intimately associated with the malignant cells.



| Tumor | Usual age at diagnosis | Common primary site | Radiographic appearance | Distinctive features | Clinical course |
|---|---|---|---|---|---|
| Fibrosarcoma | Second–sixth decade | Similar to osteosarcoma | Usually lytic | Spindle cells and collagen but no osteoid | Similar to osteosarcoma |
| Chondrosarcoma | Third–seventh decade; usually older adults | Most in trunk (especially pelvis) and proximal limbs | Bone destruction and fluffy calcification | Prolonged history of symptoms—and healthy tissue is fully developed cartilage without tumor osteoid | Metastases rare but may appear many years later |
| Osteosarcoma<br>Conventional (osteoblastic, chondroblastic, fibroblastic)<br>Telangiectatic | Second and third decade | Around knee joint and shoulder (see Fig. 35-1) | Variable, depending on degree of mineralization of osteoid (see text) | Tumor osteoid present; variable degrees of osteoblastic, chondroblastic, and fibroblastic differentiation (see text) | Early dissemination to lungs a skeleton |
| Telangiectatic | Second and third decade | Similar to conventional osteosarcoma | Predominantly lytic lesion with little or no sclerosis | Cystic, cavity-like tumor; blood-filled spaces in tumor | Similar to conventional osteosarcoma |
| Small cell | Second and third decade | Similar to conventional osteosarcoma | May be predominantly lytic | May be confused with Ewing's sarcoma | Similar to conventional osteosarcoma; radiosensitive |
| Multifocal | — | Synchronous involvement of multiple bones | Multiple skeletal sites showing densely sclerotic lesions | "Multiple primary tumors vs. metastatic primary tumor | Uniformly fatal |
| Parosteal (juxtacortical osteosarcoma) | Third decade or older | Posterior aspect of distal femur | Arises from cortex; encircles involved bone; pronounced ossification | Low-grade tumor with characteristic radiograph and pathology (see text) | Indolent clinical course with low propensity for metastasis |
| Periosteal (juxtacortical chondrosarcoma) | Fifth–seventh decade | Tibia and femur | Tumor located superficially in cortex | Tumor limited to periphery of cortex | Intermediate prognosis |

**TABLE 35-1. THE FAMILY OF OSTEOGENIC SARCOMAS**

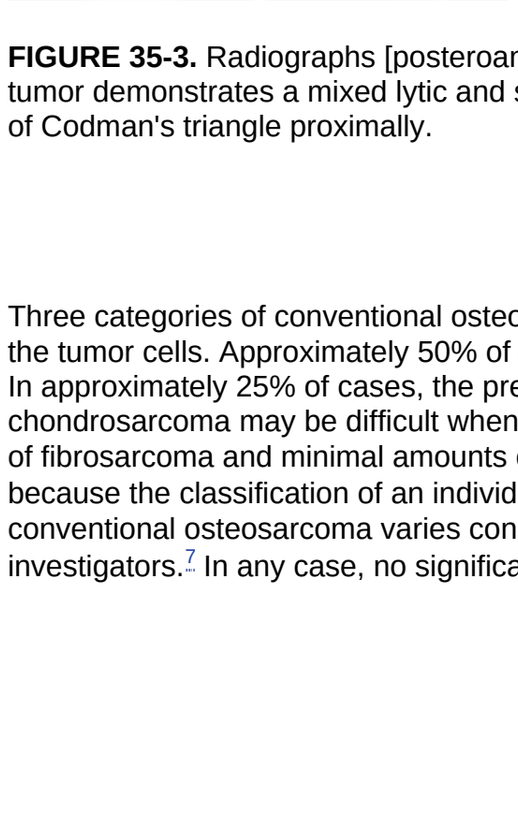


**FIGURE 35-3.** Radiographs [posteroanterior **(A)** and lateral **(B)** projections] of a conventional osteosarcoma involving the distal femur and extending up the shaft. The tumor demonstrates a mixed lytic and sclerotic appearance, a soft tissue mass with ossification apparent in the soft tissue, and periosteal reaction and the formation of Codman's triangle proximally.

Three categories of conventional osteosarcoma have been defined by Dahlin and Coventry [6,8] and Dahlin and Unni[9] on the basis of the predominant differentiation of the tumor cells. Approximately 50% of cases of osteosarcoma are characterized by abundant production of osteoid and are classified as osteoblastic osteosarcoma. In approximately 25% of cases, the predominant differentiation is toward cartilage (chondroblastic osteosarcoma), and distinguishing these tumors from pure chondrosarcoma may be difficult when osteoid production is minimal. The remaining cases demonstrate a spindle-cell stroma, with a herringbone pattern reminiscent of fibrosarcoma and minimal amounts of osteoid (fibroblastic osteosarcoma). The value of this subclassification of conventional osteosarcomas is not well established because the classification of an individual tumor is necessarily arbitrary and subject to errors of sampling. [9] The fact that the distribution of these subtypes of conventional osteosarcoma varies considerably in other series suggests that the criteria for distinguishing among these subtypes are not accepted by all investigators.[7] In any case, no significant differences in behavior or outcome can be determined among these subclasses. [8] Grading of osteosarcomas is difficult, but

the majority is judged to be high-grade.

Several variants of osteosarcoma that have been described appear to behave clinically like conventional osteosarcoma and should be treated as such. Telangiectatic osteosarcoma is an unusual variant (approximately 3% of all osteosarcomas) that characteristically appears as a purely lytic lesion on plain radiographs, with little calcification or bone formation.[2,6] The radiographic appearance is reminiscent of aneurysmal bone cyst[75] and giant-cell tumor, with which it often is confused. These tumors are grossly cystic and histologically demonstrate dilated spaces filled with blood and necrotic tissue, with viable tumor confined to the periphery of the lesion. Although sometimes reported to be associated with a particularly unfavorable outcome,[9,76] telangiectatic osteosarcoma is best approached as a conventional osteosarcoma, because recent results fail to demonstrate any difference in prognosis for patients with this histologic variant.[77,78 and 79] Small-cell osteosarcoma[80,81,82,83,84 and 85] is a variant that histologically is confused easily with Ewing's sarcoma. The production of malignant osteoid matrix distinguishes the tumor from Ewing's sarcoma, a critical distinction because the therapeutic approaches to the two tumors are quite different. Confusion with Ewing's sarcoma is particularly likely when tumor osteoid is not evident in the histologic sections from a small biopsy. In these cases, radiographic features—particularly the presence of tumor new bone—may reveal the true nature of the lesion and exclude the diagnosis of Ewing's sarcoma.[82] Immunocytochemical studies may be necessary to make the distinction, and recently described antibodies to the MIC2 gene product uniformly expressed by the cells of Ewing's sarcomas may be useful for this purpose.[86] Whether small-cell osteosarcoma is responsive to radiotherapy has been debated,[80,81] so that specific recommendations regarding the management of the primary tumor remain uncertain. The natural history of small-cell osteosarcoma has not been defined clearly, but little evidence suggests that this variant behaves differently from other varieties of conventional osteosarcoma.[80,81,82,83,84 and 85] An aggressive approach with systemic chemotherapy seems advised. The malignant fibrous histiocytoma (MFH) subtype of osteosarcoma[87] is distinguished from primary MFH of bone[9] on the basis of the finding of tumor osteoid and woven bone in the osseous component (but not the extraosseous component) of the tumor.[87] Although a rare variant of osteosarcoma, the MFH subtype accounts for one-third of osteosarcomas in patients older than 60.[88] Both the MFH variant of osteosarcoma and primary MFH of bone appear to behave clinically as high-grade sarcomas, and the distinction may be academic.[87]

Certain variants of osteosarcoma have been distinguished from conventional osteosarcoma because of their unique clinicopathologic features and characteristic clinical behavior. The most important of these clinicopathologic variants is the parosteal osteosarcoma (juxtacortical osteosarcoma), which constitutes fewer than 5% of all osteosarcomas.[4,9,89,90 and 91]The posterior aspect of the distal femur is the site most commonly involved, but other long bones may be affected. Clinically, these lesions occur in older patients with a relatively long history of symptoms (sometimes longer than 1 year). Parosteal osteosarcoma also presents a characteristic radiographic appearance (Fig. 35-4A,Fig. 35-4B); the tumor appears to arise from the cortex from a broad base without invasion of the medullary cavity, a finding that can be confirmed by computed tomography (CT) or magnetic resonance imaging (MRI), and the lesion encircles the involved bone. Intense ossification is typical and, histologically, these lesions appear to be low-grade. In contrast to classic osteosarcoma, parosteal osteosarcomas are clinically indolent and are characterized by local recurrence rather than by distant metastatic spread after inadequate surgical excision. The outcome in patients who undergo radical excision of the primary tumor usually is favorable.[4,9,89,90] A substantial proportion of parosteal osteosarcomas are found to have undergone dedifferentiation (i.e., a high-grade spindle-cell sarcoma coexists with a lower-grade, more typical parosteal sarcoma).[91] Dedifferentiation is seen more often at the time of a recurrence but is observed in more than 50% of cases at initial diagnosis.[91] Rarely, lesions appearing to be parosteal osteosarcomas have been encountered that are high-grade or appear to invade the medullary cavity. Medullary cavity invasion is more common in those tumors that exhibit dedifferentiation.[91] Although such features may not be anticipated from the clinical or plain radiographic features, medullary invasion sometimes is demonstrable by MRI or CT. Such lesions are much more ominous and behave aggressively, in the manner of classic high-grade osteosarcomas, with a high propensity for metastatic spread.

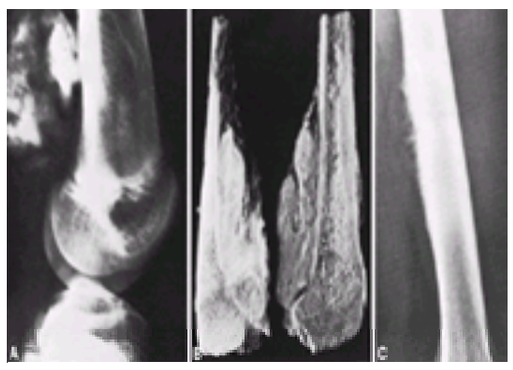


**FIGURE 35-4. A:** Radiograph of a typical parosteal osteosarcoma involving the posterior aspect of the distal femur. Intramedullary involvement is not evident. **B:** Sagittal section of gross specimen from a parosteal osteosarcoma (not the same case as in **A**). The bony mass is adherent to the underlying cortex and is situated entirely on the surface of the bone. **C:** Radiograph of a typical periosteal osteosarcoma. Tumor involving the midshaft of the femur is radiolucent, does not involve the marrow, and shows perpendicular spiculation. (Reproduced with permission from DC Dahlin, KK Unni. Osteosarcoma of bone and its important recognizable varieties. Am J Surg Pathol 1977;1:61–71.)

Another rare variant is the periosteal osteosarcoma[92,93] that, like parosteal osteosarcoma, arises on the surface of bone without involvement of the marrow cavity. The lesion occurs frequently in the second decade of life and has a propensity for involvement of the upper tibial metaphysis, appearing as an ill-defined radiolucent lesion on the surface (Fig. 35-4C). Histologically, the tumors are relatively high-grade, predominantly chondroblastic osteosarcomas and must be distinguished from periosteal chondrosarcoma, a lower-grade tumor that does not have tumor osteoid and follows a more indolent course. The prognosis for patients with periosteal osteosarcoma is worse than that for those with parosteal osteosarcoma; the tumors tend to recur locally unless radical resection is performed.[9,92,93] Lesions of the femur are more likely to recur, even after approaches that accomplish wide (but not radical) surgical margins.[93] Some authorities contend that periosteal osteosarcomas may, indeed, represent high-grade surface lesions presenting early in their clinical course.[2] This theory might explain the intermediate prognosis for patients with periosteal osteosarcomas and the higher rate of systemic spread observed. In this view, systemic chemotherapy (as for children with conventional high-grade osteosarcoma) is indicated for patients with periosteal osteosarcomas as well, but this area remains controversial because such lesions are rare. It should be noted that conventional high-grade osteosarcomas may develop on the surface of bone and may be confused with parosteal or periosteal osteosarcoma.[94,95] However, high-grade osteosarcoma of the surface of bone resembles conventional osteosarcoma histologically and in its clinical behavior. Low-grade intraosseous tumors also occur,[96] although they are rare. Intraosseous low-grade osteosarcomas are well-differentiated, with minimal cytologic atypia, and can be mistaken for benign conditions, particularly fibrous dysplasia. A tendency to local recurrence, especially after inadequate surgery, is the rule, and distant metastases are unusual.[9,96] Critical considerations are the grade of the lesion and its anatomic location. High-grade tumors require systemic chemotherapy, whether they arise in the conventional medullary location or on the surface of a bone. Low-grade lesions should be treated by wide surgical resection without adjuvant chemotherapy.

Several additional variants of osteosarcoma are distinguished from classic osteosarcoma because of differences in biologic behavior. Primary osteosarcoma of the jaw occurs most often in older patients, tends to demonstrate prominent chondroid differentiation, and is associated with a more indolent course, having a tendency to local recurrence rather than to distant metastases, especially if the tumors are low-grade.[97,98] A recent review of published series of osteosarcoma of the head and face suggests that osteosarcoma patients benefit from the addition of systemic chemotherapy.[99] By contrast, osteosarcoma occurring in patients with Paget disease is associated with a very aggressive clinical course and few survivors.[9] Histologically, however, the tumors are identical to conventional osteosarcoma, as are osteosarcomas occurring in irradiated bones. Extraosseous osteosarcoma is an uncommon variant that arises outside of bone and occurs most frequently in the soft tissues of the lower extremity in middle-aged adults.[100,101] Extraosseous osteosarcomas are seen (although not exclusively) as a late complication of radiotherapy. Local excision of these lesions is inadequate treatment because local recurrences and distant metastases invariably follow limited surgery.[100,101] Finally, multifocal osteosarcoma is a rare entity in which multiple synchronous skeletal tumors are present at diagnosis and in which each lesion resembles a primary tumor radiographically, suggesting a multicentric origin of the sarcoma. Not clear is whether such sarcomas arise in multiple sites or whether one of the lesions is the true primary tumor that has spread rapidly to other skeletal sites in the absence of lung metastases.[9,102,103] Interestingly, a study of four patients with multifocal osteosarcoma revealed germline mutations of the p53 gene in two cases.[104]

## CLINICAL PRESENTATION, NATURAL HISTORY, AND PATTERNS OF SPREAD

The majority of patients with osteosarcoma present with pain over the involved area, with or without an associated soft tissue mass. The average duration of symptoms is 3 months, although a history of 6 months or longer is not uncommon. Parosteal osteosarcomas, in particular, can be associated with painful symptoms of several years' duration, reflecting the relatively indolent behavior of this variant. Osteosarcoma characteristically involves the long tubular bones, especially adjacent to the knee joint. The distal femur and proximal tibia are the sites involved most frequently, followed in decreasing frequency by the proximal humerus and middle and proximal femur (Fig. 35-1).[2,6] Involvement of the flat bones of the axial skeleton, notably the pelvis, occurs in approximately 15% to 20% of cases but accounts for fewer than 10% of cases in the pediatric age group. Osteosarcoma of the jaw is a peculiar entity that accounts for fewer than 7% of all osteosarcomas and follows a more indolent course, as noted.

Approximately 15% to 20% of patients with osteosarcoma present with visible macrometastatic disease. The majority of these metastatic lesions are found in the lungs, although a small fraction of patients present with bone metastases with or without concomitant pulmonary metastases. Presentations with multiple bone metastases may reflect multifocal primary tumors (multifocal sclerosing osteosarcoma) and carry an extremely grave prognosis. [9,102,103]

Because osteosarcoma has been demonstrated to be relatively unresponsive to radiotherapy, surgical removal of the primary tumor is necessary for durable local control (as discussed in the section Treatment). Fortunately, the majority of patients with osteosarcoma present with primary tumors of the extremities, and local control can be achieved readily by amputation (see Chapter 12). In historical series (before 1970), the outlook for children with osteosarcoma was dismal, despite adequate surgical control of the primary tumor by amputation.[105,106,107,108 and 109] The overwhelming majority of patients presenting without evidence of metastases and treated only with surgery of the primary tumor ultimately developed distant metastases; 50% of patients developed metastases—virtually always in the lung—within 6 months of amputation and, overall, more than 80% ultimately developed recurrent disease. This discouraging natural history was documented at a number of centers and was summarized in a literature review in 1972.[109] Eleven studies conducted between 1946 and 1971 reporting data from 1,337 patients were reviewed. Of 1,286 patients with adequate follow-up treated with surgical ablation of the primary tumor (almost all amputations), only 253 patients (19.7%) survived 5 years. The 10-year survival dropped only slightly, to 16%, indicating that the majority of 5-year survivors were cured.

The appearance of metastatic disease was an ominous sign in historical series because few patients survived beyond 1 year after detection of tumor recurrence. The majority of patients developed multiple bilateral pulmonary metastases as the first evidence of recurrence, [4,110] and more than 95% of patients who died of metastatic disease had lung involvement at the time of death. Metastases to bones of the skeleton occur in 15% to 30% of patients. Less common sites of metastases occurring preterminally or discovered at autopsy include pleura, pericardium, kidney, adrenal gland, and brain. [108] Involvement of lymph nodes is unusual but is a poor prognostic sign. Death from metastatic disease results from pulmonary failure due to widespread lung metastases, pulmonary hemorrhage, pneumothorax, and superior vena cava obstruction.

Although variability in overall outcome of patients with osteosarcoma was noted in historical series, the expectation that fewer than 20% of patients would survive beyond 5 years (based on data from multiple centers) served as the background for trials of adjuvant chemotherapy conducted in the 1970s and 1980s. The inescapable conclusion from those historical studies was that 80% of patients presenting without overt metastatic disease in fact had microscopic subclinical metastases at the time of diagnosis that were undetectable by techniques then available.

By the late 1970s, the prognosis for children with osteosarcoma clearly was improving, an improvement largely attributed to the application of adjuvant therapies. However, data from the Mayo Clinic[111,112 and 113] suggested that the natural history of osteosarcoma had changed since the late 1960s and that the prognosis for patients had improved independently of the administration of adjuvant therapy. Data from other small studies, [114,115 and 116] particularly from a randomized controlled trial of adjuvant chemotherapy with single agent high-dose methotrexate conducted at the Mayo Clinic, [117] appeared to confirm this change in the natural history of osteosarcoma. Thus, at the beginning of the 1980s, the true natural history of osteosarcoma was controversial, and assessing the efficacy of adjuvant therapy was difficult. In the 1970s, trials of adjuvant chemotherapy for osteosarcoma were conducted without concurrent control groups, and benefits were inferred by comparison to historical controls and to the expectation that fewer than 20% of patients treated only with surgery would survive relapse-free. The controversy surrounding the apparent change in the natural history of osteosarcoma and its impact on the interpretation of adjuvant therapy trial results cast doubt on the apparent benefits of adjuvant chemotherapy demonstrated in numerous uncontrolled trials of the 1970s. [118,119 and 120]

Two trials conducted in the 1980s were designed to address the natural history of surgically treated osteosarcoma of the extremity. [121,122 and 123] The outcome of patients in these trials, who were treated only with surgery of the primary tumor, recapitulated the historical experience prior to 1970: More than one-half of these patients developed metastases within 6 months of diagnosis and, overall, more than 80% developed recurrent disease within 2 years of diagnosis. Thus, the natural history of osteosarcoma apparently has not changed over time, and fewer than 20% of patients treated only with surgery of the primary tumor can be expected to survive relapse-free. The bleak historical experience that had served as the background for numerous uncontrolled adjuvant trials of the 1970s appears to be an equally valid control for studies of the 1980s, 1990s, and beyond.

## CLINICAL EVALUATION AND DIAGNOSTIC STUDIES

The evaluation of suspected osteosarcoma begins with history recording, physical examination, and plain radiographs. The duration of symptoms is variable and may have prognostic significance (see the section Prognostic Factors). Pain in other bony sites may represent metastatic involvement, but metastases are most likely to occur in the lungs and do not produce respiratory symptoms in the absence of extensive lung involvement. Systemic symptoms, such as fever and weight loss, occur rarely in the absence of very advanced disease. [4,8] Physical examination typically is remarkable only for the soft tissue mass usually evident at the primary site; regional and distant lymph node metastases rarely are observed.

Laboratory evaluation seldom is revealing. Elevation of the serum alkaline phosphatase level is observed in more than 40% of patients but does not correlate reliably with disease extent,[4,124] although the serum alkaline phosphatase level may have prognostic significance (see the section Prognostic Factors). The serum lactate dehydrogenase (LDH) level may be elevated in approximately 30% of patients presenting without metastases. [122]

### Radiologic Evaluation

Radiographic examination of the involved bone is extremely useful in the evaluation of patients with a suspected malignant bone tumor (see Chapter 9). Plain films of malignant bone tumors usually reveal permeative destruction of the normal trabecular pattern, with indistinct margins and no endosteal bone response. Intense periosteal new bone formation and lifting of the cortex with formation of a Codman triangle are common (Fig. 35-3).[125] A soft tissue mass frequently is observed as the tumor erodes from the medullary cavity through the cortex and into the adjacent soft tissue. Characteristic radiographic features, along with clinical information and tumor location, permit prediction of the histologic diagnosis from the plain radiographs in more than two-thirds of cases of osteosarcoma. [125]

Osteosarcomas of the long bones invariably involve the metaphyseal portion of the bone; involvement of the diaphysis of the long bones occurs in fewer than 10% of the cases.[126] Thus, the eccentric location of the tumor in the metaphyseal portion of the long bone is characteristic of osteosarcoma, whereas Ewing's sarcoma (the most frequent consideration in the differential diagnosis) tends to occur in the flat bones or in the diaphyseal portions of the long bones of the skeleton and more frequently appears as a predominantly lytic lesion on plain radiographs. Ossification in the soft tissue in a radial or “sunburst” pattern is classic for osteosarcoma but is neither a reliable nor a specific feature. None of the radiographic features is pathognomonic, however, and biopsy always is required to confirm the diagnosis. Radiographically, osteosarcomas may appear to be osteosclerotic in approximately 45% of cases, purely osteolytic in 30%, and mixed sclerotic and lytic in the remaining 25%.[125] Such radiographic findings reflect the degree of ossification and mineralization rather than the amount of osteoid found in the tumor, because uncalcified osteoid substance produces no radiopacity even if present in large amounts. [9]

Although plain radiographs are extremely useful in evaluating malignant bone tumors, the extent of the primary lesion often is underestimated seriously by such studies. Further radiographic evaluation of the primary tumor thus is essential in planning the definitive surgery. Both CT and MRI contribute substantially to the accurate estimation of local tumor extent before surgery. MRI is particularly suitable for assessing bone tumors and has largely supplanted the CT scan for this purpose (Fig. 35-5).[127,128,129,130 and 131] Tumor extent as defined by MRI has been found to be an accurate estimate of tumor boundaries determined at the time of definitive surgery.[128] MRI has been particularly accurate in assessing the intraosseous extent of tumor and tumor extent with respect to muscle groups, subcutaneous fat, joints, and major neurovascular structures around the tumor.[131] Such information is critical in planning the level of amputation or for planning limb salvage resections. The use of gadolinium-diethylenetriamine penta-acetic acid (Gd-DTPA) as an agent for the purpose of enhancing the contrast of bone tumors on MRI presents some theoretic advantages, but preliminary data are disappointing, and Gd-DTPA enhancement does not contribute significantly to the assessment of tumor

extent.[131]

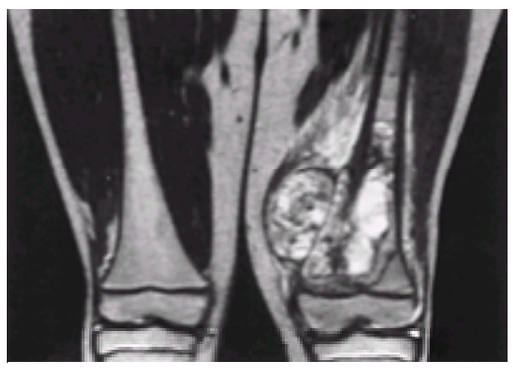


**FIGURE 35-5.** T2-weighted coronal magnetic resonance image through both distal femurs of the patient whose plain radiographs are shown in Figure 35-3 demonstrates the large distal femoral osteosarcoma with extensive soft tissue invasion. The proximal extent of tumor in the marrow cavity is clearly demonstrated.

The radionuclide bone scan also is indicated in the initial diagnostic evaluation to define the extent of the primary tumor. Because uptake of the radiopharmaceutical will extend slightly beyond the limits of tumor, it defines a safe margin to use in planning surgery of the primary lesion. [132,133] The sensitivity of radionuclide bone scanning is useful also in the detection of "skip lesions," which are seen infrequently in patients with osteosarcoma. [134] Increased uptake of a radiopharmaceutical in a site separate from the primary tumor but in the involved bone should alert the surgeon to the possible existence of skip lesions, information that should be considered in planning the surgical approach to the tumor.

In the past, arteriography was believed to be useful for evaluating the primary tumor for consideration of limb salvage procedures. However, with the advent of MRI, most investigators find that angiograms contribute little in determining local operability (see Chapter 9). Arteriograms performed in only the anteroposterior plane provide little definitive information on the relationship of the extramedullary tumor extent to the primary vascular structures. Arteriograms also provide poor visualization of veins, which may be a more significant surgical problem than arteries.

The presence of metastases at diagnosis is an extremely important prognostic variable with a major impact on management, so appropriate surveillance must precede the definitive approach to the primary tumor. The lung is the first site of metastasis in 90% of children with osteosarcoma, and routine posteroanterior and lateral radiographs of the chest allow detection of metastases in the majority of cases. Linear tomography and CT of the chest are more sensitive in detecting pulmonary metastases: In as many as 10% to 20% of patients, metastatic nodules found on linear tomography or CT of the chest are undetectable on conventional chest radiographs.[135] Because CT is more sensitive than linear tomography, especially for the detection of pleural-based lesions, [125,136,137 and 138] the CT scan probably is the choice for screening osteosarcoma patients for metastatic disease, although false-positive results have been a problem with this technique. If on CT scan a lesion cannot be defined unequivocally as metastatic disease, histologic confirmation is indicated, especially if the lesion does not appear on plain radiographs.

Because metastases to other bones of the skeleton are seen in approximately 10% of patients with osteosarcoma, a radionuclide bone scan also is indicated in screening for metastatic disease. Scanning with methylene diphosphonate labeled with technetium $^{99m}$TC has been found to be very sensitive for the detection of metastatic bony sites and, in the majority of cases, is more sensitive than are plain radiographs. [132,139]

**Follow-Up Studies**

With the increasing use of chemotherapy administered before definitive surgery to facilitate limb-sparing operations, radiographic evaluations have assumed increasing importance for early assessment of responsiveness of the primary tumor to chemotherapy and for predicting the histologic response (discussed in the sections Limb Salvage Surgery and Presurgical Chemotherapy). Several techniques have been proposed for this purpose, but none has been reported thus far to be sufficiently sensitive or reliable. Assessment by conventional radiographs, CT, and MRI show definite changes in response to presurgical chemotherapy, but the changes do not correlate reliably with histologic response, and the radiographic assessment is necessarily subjective and nonquantitative. [131] Investigators in the German Society for Pediatric Oncology (GPO) have used scintigraphic evaluation with $^{99m}$Tc–methylene diphosphonate parametric imaging of the tumor blood pool and labeled methylene diphosphonate plasma clearance by the tumor bone. Presurgical scintigraphic evaluation of tumor response to presurgical chemotherapy correlated highly with the degree of tumor regression as graded histologically after surgery. [140] Studies have indicated that response in the primary tumor measured by thallium 201 scintigraphy more reliably predicts the histologic tumor response. [141] Arteriography has been recommended by some investigators (particularly those who favor the administration of repeated courses of intra-arterial chemotherapy), because areas of tumor vascularity and tumor stain can be assessed repeatedly with each course of chemotherapy. However, results of angiography have not been fully reliable for distinction between responders and nonresponders. [142] Assessment by static MRI has been disappointing. However, dynamic MRI using bolus injections of Gd-DTPA may prove to be a promising technique for following responsiveness of primary tumors.[143] Positron emission spectroscopy would appear to be ideally suited, but the technique is not yet available at all centers.

Radiographic evaluations also are a critical component of the longer-term follow-up of patients treated with surgery and chemotherapy as surveillance for metastatic disease and local recurrence. Patients with osteosarcoma should be followed up frequently with radiographic studies for metastatic surveillance for at least 5 years from the completion of therapy. Because the overwhelming majority of first recurrences appear asymptomatically in the lungs, plain chest radiographs should be performed monthly for at least 2 years from diagnosis and with decreasing frequency thereafter. The importance of detecting metastases early cannot be overemphasized (see the section Treatment of Metastases). Thus, any abnormality detected on plain chest radiographs should be followed up diligently. CT of the chest is a useful adjunct to plain radiographs for this purpose and should be performed routinely every 4 to 6 months for the first 2 years from diagnosis and whenever a persistent suspicious abnormality appears on plain films. Radionuclide bone scans also should be obtained every 4 to 6 months for the first 2 years. Although bone metastases are likely to produce symptoms, occasional asymptomatic metastases in the skeleton may be detected by bone scan, and any abnormality should be confirmed by plain radiographs. Evaluation of the stump after amputation and of the affected limb after tumor resection can be difficult because of bone scan and radiographic abnormalities resulting from unequal weight bearing and callus formation. However, evaluation of the primary site should not be overlooked because of the possibility of local recurrence, especially after limb salvage procedures.

**Biopsy**

Although the radiographic findings in a patient with a suspected malignant bone tumor may be highly suggestive, a biopsy is always required to confirm the diagnosis. The timing of the biopsy in relation to the other diagnostic procedures has been debated; many investigators claim that interpretation of the bone scan and MRI (in terms of the extent of the primary tumor) may be unreliable if these investigations are undertaken after biopsy. [125,131,144] The tissue disturbance caused by the biopsy in most instances should be minimal, especially if a needle biopsy is performed, and should not have a major impact on information from radiographic studies. A major advantage of obtaining the imaging studies prior to the biopsy, however, is that the surgical approach to resection of the primary tumor can be considered, and the biopsy incision can be placed in a location that allows excision of the site en bloc with the tumor specimen. Because many biopsies in children are performed with general anesthesia, obtaining the chest CT scan prior to the biopsy reduces the likelihood that postoperative atelectasis will be confused with a pulmonary metastasis.

The biopsy should be performed or directed by an orthopedic surgeon familiar with the management of malignant bone tumors and experienced in the required techniques,[145] preferably by the surgeon who ultimately will perform the definitive surgical procedure. Fine-needle aspiration and core-needle biopsy have been recommended at a number of centers,[146,147,148,149 and 150] but most patients require open biopsy to be certain that a generous sample of adequate and representative tissue is obtained. Many pathologists are reluctant to render a definitive diagnosis when only small fragments of tissue are available. Moreover, if additional tumor tissue is obtained, it may be submitted for other biologic studies, some of which (e.g., DNA content, genetic studies, and MDR determination) may have important prognostic significance (see the section Prognostic Factors).[151,152,153,154,155,156,157 and 158] The surgeon should plan the biopsy carefully, with consideration of subsequent definitive surgery (or radiotherapy), because a poorly conceived and poorly placed biopsy may jeopardize the subsequent treatment, especially a limb salvage procedure.[159,160 and 161]

The incision should be made longitudinally in a fashion that disturbs as little of the normal anatomy as possible. Having both plain radiographs and the MRI available is helpful to determine the most direct route between the skin and the primary tumor. In general, the incision is made over the soft tissue mass. Making a surgical defect in the bone is not necessary if tissue can be obtained from the soft tissue mass. Avoiding a bone defect renders subsequent fracture less likely, and the least mineralized tissue is at the periphery of the tumor. A frozen section taken at the time of biopsy ensures that adequate tissue has been obtained for diagnosis. Several studies have indicated that the frequency of tumor seeding of biopsy sites is high, and so, even if it is a needle biopsy, the biopsy site must be excised en bloc with the tumor during the definitive surgical procedure. [162,163] Placement of the biopsy site with a view to the eventual definitive surgical procedure is essential (especially if limb salvage surgery is a consideration) to allow excision of the biopsy site in continuity with the definitive bony resection. Meticulous hemostasis after the biopsy also is essential. Placement of absorbable gelatin sponges or methyl methacrylate in the bone defect is useful to control bleeding, and efforts to control muscular vessels with external compression for 1 to 2 days may reduce the incidence of hematomas and subsequent wound infection in the biopsy site. [159,160,164] The use of a tourniquet is optional, whereas obtaining adequate hemostasis and using drains brought out near or through the incision prior to closure to prevent a large hematoma are important.

In the past, showering of tumor cells at the time of biopsy was believed to result from surgical manipulation and to account for the poor prognosis for patients with osteosarcoma. Reducing the dissemination of tumor cells was thought possible by performing the definitive surgical procedure at the same time as the initial biopsy. However, this strategy of biopsy and definitive surgery during one operation has become less popular for a number of reasons: Immediate definitive surgery after the biopsy does not lead to a superior outcome; the difficulties of making the histologic diagnosis from the frozen section alone are significant; psychological advantages accrue in preparing the patient for definitive surgery; the surgeon may benefit from a longer interval to plan the definitive surgery (particularly if a limb salvage is undertaken); and the administration of chemotherapy before definitive surgery for osteosarcoma has gained support. In most cases, the biopsy is performed to confirm the diagnosis, and definitive surgery of the primary tumor is undertaken at a later date, in a separate surgical procedure.

## Staging

Because of the unsuitability of standard staging systems when applied to skeletal tumors, Enneking et al. [165,166] at theUniversity of Florida established a staging system for malignant skeletal tumors based on a retrospective review of cases of primary malignant tumors of bone treated by primary surgical resection. This system categorizes nonmetastatic malignant bone tumors by grade—low-grade (stage I) or high-grade (stage II)—and further subdivides by the local anatomic extent: intracompartmental (A) or extracompartmental (B). The compartmental status is determined by whether the tumor extends through the cortex of the bone. Patients with distant metastases are categorized as stage III. The classification was designed primarily for determining surgical treatment and comparing results between institutions and surgeons. High-grade intramedullary lesions (i.e., stage IIA) are very rare, because most high-grade tumors (including osteosarcomas) break through the cortex into the extramedullary tissues early in their natural history and present as stage IIB lesions. The American Joint ommittee on Cancer has a similar staging system for bone tumors; it constitutes four instead of three stages, but stage III is undefined. It is similar to the tumor-node-metastasis (TNM) system used for other cancers but is essentially the same as the Musculoskeletal Tumor Society system. Neither staging system has been subjected to tests of validity. [167] Nevertheless, the Enneking staging system remains the only one that is widely accepted for skeletal tumors, and its use facilitates comparison of outcomes of different therapeutic regimens.

## Prognostic Factors

Several clinical characteristics are thought to be of prognostic significance for patients with osteosarcoma, independent of therapy. [168] Such factors may become less powerful prognostically as the outcome for patients with osteosarcoma continues to improve. The most important prognostic factor appears to be extent of disease at diagnosis, in that patients with overt metastatic disease have an unfavorable outcome. Although aggressive approaches to disease in patients presenting with metastases have improved their prognosis somewhat (discussed in the section Treatment of Metastases), the majority of such patients ultimately die of their disease.

Histology exerts an important influence on outcome. Parosteal and intraosseous well-differentiated osteosarcomas, in particular, are associated with a favorable prognosis, and periosteal osteosarcomas have an intermediate outlook. [89,90,91,92 and 93] Among the variants of conventional osteosarcoma, there appears to be no significant relation between histologic subtype (osteoblastic, fibroblastic, and chondroblastic) and overall survival. [8] Telangiectatic osteosarcomas have been associated with a particularly poor prognosis in some series, [9,76] but the behavior of this variant in the era of chemotherapy appears to be similar to that of other conventional osteosarcomas. [77,78 and 79]

Osteosarcomas arising in preexistent lesions or from radiation exposure behave clinically as de novo lesions, except those in patients with Paget disease, which are associated with an adverse prognosis. [9] Histologic grading likewise has not correlated reliably with outcome, in large part because most classic osteosarcomas are high-grade lesions. DNA content of tumor cells has been investigated in patients who had conventional osteosarcoma and received adjuvant chemotherapy, and it has been found to correlate significantly with outcome. [156] Patients with near-diploid tumor cell lines fare significantly better than do those with hyperdiploid tumor cell lines. Therefore, measurement of tumor DNA content may be extremely useful in identifying high- and low-risk cases.

The primary site of disease also is an important variable. A review of survival figures from several large series [169,170] suggests that patients with axial skeleton primary tumors have a poor prognosis. Because complete surgical excision with clean margins is a prerequisite for long-term disease control, tumors arising in certain axial skeleton sites (e.g., skull, vertebrae) are not amenable to curative surgery. Now, more innovative surgical strategies are used for tumors in some axial skeleton sites (particularly primary tumors in the pelvis), and tumors once considered unresectable can be approached with curative intent. Less intuitive is the association of tibial primary lesions with a more favorable prognosis in most, [122,169,171] but not all, [4] series. Tumors of the proximal humerus have been associated variably with a favorable or unfavorable prognosis. In general, more distal primary tumors have been associated with a more favorable prognosis.

Tumor size also has been cited as a powerful prognostic factor. In the prechemotherapy era, small tumors (less than 5 cm) were associated with a favorable prognosis and large tumors (greater than 15 cm) with a dismal prognosis. [4] This relation has not been confirmed in all series, and the effect of tumor size may result from a correlation of size and primary site. [170] In studies from the GPO, tumor involvement of more than one-third of the affected bone was associated with adverse outcome. [172] Data from an other study indicate that the local extent of tumor predicts outcome more reliably than does tumor size alone. [173] Among patients with clinical stage IIB lesions, those with tumors that penetrated the periosteum and invaded two or more structures adjacent to the bone were much more likely to suffer treatment failure than were patients with less extensive tumors. Intramedullary extent of tumor (as evaluated by CT scan) also correlates with the risk of subsequent development of metastatic disease. [174] Skip metastases, although rare, are an ominous sign: Almost all patients with skip metastases in one series had either local recurrence or distant metastases, [175] despite treatment with adjuvant chemotherapy. These data suggest that skip lesions from osteosarcoma are best considered to be metastatic lesions and to be associated with a poor outcome.

Duration of symptoms before treatment may be an indirect measure of tumor growth rate and has prognostic value. Patients with longer durations of symptoms have a superior outcome. [4] In addition, patients with long intervals between the onset of symptoms and diagnosis are likely to have an indolent variant of osteosarcoma (particularly parosteal osteosarcoma).

Additional patient characteristics reported to be of prognostic value include age (children younger than 10 years fare worse; patients older than 20 years have a more favorable outlook); gender (female patients have a more favorable outcome) [176,177]; and serum and tumor tissue alkaline phosphatase (elevated levels are associated with a greater risk of subsequent metastatic disease). [124,178] The level of serum LDH also has been found to be an important prognostic factor in patients treated with adjuvant chemotherapy. [122] In the Multi-Institutional Osteosarcoma Study, elevated serum LDH at diagnosis was found to be the most powerful, single, adverse prognostic factor for patients with nonmetastatic osteosarcoma of the extremity treated with adjuvant chemotherapy. In studies from the Memorial Sloan-Kettering Cancer Center, [171] a steady increase in risk of relapse or death was associated with increased serum LDH activity at diagnosis. These findings parallel results of studies of non-Hodgkin's lymphoma, in which serum LDH has been found to correlate with both tumor burden and prognosis. [179]

Recently, attention has been directed at molecular markers of prognosis. A study [180] of a small number of patients with osteosarcoma examined loss of heterozygosity (LOH) at the *RB* gene locus and found that patients whose tumors demonstrated no LOH at *RB* were less likely to present with metastatic disease and that all such patients were projected to survive without recurrence at 5 years. The prognosis for patients with LOH at *RB* was significantly worse, even if only patients without evidence of metastasis at diagnosis were considered. Expression of the human epidermal growth factor receptor 2 (HER2/erbB-2) has been shown to have prognostic significance in a number of human cancers. In patients with osteosarcoma, overexpression of HER2 has been associated with an inferior outcome. [181,182]

Response to preoperative chemotherapy is another powerful prognostic indicator [168,183,184 and 185]; however, tumor response is not known prospectively (i.e., before the institution of therapy). When an experienced pathologist carefully evaluates the resected specimen, the proportion of viable tumor cells surviving after presurgical chemotherapy can be determined. Several classification schemes have been developed but in general patients who demonstrate fewer than 2% residual viable tumor cells in the resection specimen have a prognosis better than those patients with higher proportions of viable tumor cells remaining. In theory, more aggressive therapy can be administered to those with a poor histologic response; however, to date no studies have demonstrated the efficacy of this approach.

Resistance to chemotherapy probably is responsible for poor outcome in some patients with osteosarcoma. The MDR1 gene encodes a glycoprotein that transgresses the cell membrane. Termed *p-glycoprotein*, this active pump mechanism actively excludes certain classes of drugs from the tumor cell and can be reversed by certain agents. Recent studies have shown that the overexpression of p-glycoprotein is associated with an unfavorable outcome in patients with osteosarcoma treated with multi-agent chemotherapy and is more predictive of event-free survival than is histologic assessment of the primary tumor after presurgical chemotherapy. [151,153] These results remain to be confirmed. Studies have been performed also to investigate mechanisms by which osteosarcoma may develop resistance to methotrexate. Osteosarcoma cells develop loss of the reduced folate carrier, and this loss is associated with a lower probability of favorable histologic response in the primary tumor after treatment with multi-agent chemotherapy, which includes high-dose methotrexate. [186]

## TREATMENT

### Surgical Management of the Primary Tumor

Because osteosarcoma usually is unresponsive to conventional-dose radiotherapy, management of the primary tumor in extremity lesions is surgical. Removal of all gross and microscopic tumor with a cuff of normal tissue completely surrounding the tumor is required to prevent local recurrence. Primary surgical procedures fall into two major categories: amputation and limb salvage procedures. Both approaches incorporate the basic principle of en bloc excision of the tumor and biopsy site through normal tissue planes. Other procedures, such as intralesional curettage or marginal resection, which often are successful for benign tumors of bone (e.g., giant-cell tumor, chondroblastoma, aneurysmal bone cyst), are inadequate for local control of osteosarcoma. Malignant tumors must be removed en bloc with a margin of normal, uninvolved tissue. Local extension of malignant bone tumors occurs primarily by intramedullary and extramedullary extension. After the tumor breaks through the cortex, it infiltrates normal muscle. However, direct invasion of adjacent arteries or nerves is rare, and involvement of veins usually is by tumor thrombus rather than by direct invasion of the vein wall. Osteosarcomas may cross the growth plate to enter the epiphysis of the bone but seldom cross the articular cartilage.[187,188,189 and 190] The adjacent joint may be involved, however, in the event of a fracture of the articular cartilage or if the tumor travels along the capsular structures or cruciate ligaments to enter the joint. [191] This observation has been used as a rationale to perform extra-articular resections in all osteosarcomas, but the advent of MRI has rendered possible the accurate assessment of the joint for tumor involvement. Because osteosarcomas seldom actually penetrate the joint, intra-articular resections are possible in the majority of extremity osteosarcomas. [192]

The selection of surgical procedure involves consideration of several interrelated factors, including tumor location, size, or extramedullary extent; presence or absence of distant metastatic disease; and such patient factors as age, skeletal development, and lifestyle preference, which may dictate the suitability of limb salvage or amputation.

### Amputation

The traditional surgical approach to local control of osteosarcoma of the extremity is amputation, which permits removal of all gross and microscopic tumor with clean margins and provides durable local control in most cases. However, amputation, even with a wide margin of normal tissue, has not prevented stump recurrence in all cases. Stump recurrence has been attributed to the extensive intramedullary spread and to the existence of skip lesions. Skip lesions—tumor deposits in the affected bone that are separated from the primary tumor by several centimeters of normal bone—have been reported to occur in up to 20% of cases of osteosarcoma. [134] This high incidence of skip lesions led some surgeons to recommend removal of the entire affected bone at the next proximal joint to ensure clean surgical margins and to avoid the problem of residual tumor in the stump. Such radical amputations result in considerable aesthetic and functional morbidity and seriously diminish the functional rehabilitation available with prostheses. Furthermore, such radical surgery has been demonstrated not to translate into better overall survival of patients. [193] The advent of more sensitive imaging techniques, such as MRI, renders possible the accurate definition of the intramedullary extent of tumor and the detection of skip lesions. This capability has enabled orthopedic surgeons to proceed confidently with cross-bone amputations to treat the primary tumor, allowing a safety margin of normal marrow above the most proximal medullary extent of tumor as defined by the MRI.[194,195,196 and 197] The local recurrence rate has been acceptably low (less than 5%), indicating that skip lesions are indeed rare. [198] A preoperative MRI of the entire involved bone should be scrutinized carefully for marrow extent and skip metastases, and the pathologist should bisect the amputated bone specimen immediately after it has been removed to ensure complete excision of the tumor. Although used in common practice, frozen sections of marrow margins have not been shown to be of much value. [199]

The treating team must have detailed discussions with affected children and parents regarding the choice between amputation and limb salvage. No clear advantage derives from either approach, although they are vastly different. The choice will depend on the age of the patient, the location of the tumor, the presence or absence of a pathologic fracture, and the desires of the patient and family. For lesions of the foot or ankle, a below-knee amputation probably is more functional than any limb salvage resection. For lesions above the knee, the advantage of amputation is less clear, and patients with high-thigh amputations or hip disarticulations and hemipelvectomies have difficulties in wearing prostheses. Very young patients may do better with amputation because of the avoidance of major limb length inequality, but this is a relative indication. Despite the development of myoelectric prostheses, no prosthesis comes close to duplicating normal hand function, so attempts to avoid amputation in the upper extremity are worthwhile. Amputees may have long-term complications of pressure sores from the prosthesis, phantom sensations, and phantom limb pain. Stump overgrowth in children with cross-bone amputations may be a difficult problem. [200,201,202 and 203] The vast majority of patients will chose limb salvage if given the option, but they must know that modern prosthetics, especially in the lower extremity, provide for excellent ability to walk and participate in a variety of sports activities. [204,205,206 and 207] The limb salvage patient will be advised to avoid most of these athletic activities.

If amputation is performed, early rehabilitation is important to restore the patient to a functional lifestyle (see Chapter 45 and Chapter 46). Improvements in the prostheses available for amputees have enhanced the functional results for these patients. [204] The functional results are excellent for patients who have had below-knee amputations, with very little measurable functional disability. Low above-knee amputations also have successful functional outcome. Studies of gait mechanics and oxygen consumption in lower-extremity amputees and in patients treated with limb salvage and rotationplasty have yielded conflicting results. Some studies show a higher energy consumption in amputees, whereas others show no difference in the groups studied. [207,208,209,210 and 211] The consensus is that energy consumption during gait is highest in above-knee amputees, intermediate in rotationplasty or arthrodesis, and lowest in mobile knee reconstructions. [203] Patients with tumors in more proximal segments of an extremity are likely to suffer more severe functional and cosmetic disability. Surprisingly, in several studies, no differences can be demonstrated in outcome vis-à-vis acceptance of their surgical procedure, ability to walk, pain, or psychosocial adjustment for patients undergoing amputation as compared to those treated with limb-sparing resection for tumors of the lower extremity. Most studies have shown that amputees adjust to their loss, although their concerns differ from those of patients who undergo limb salvage procedures; amputees are concerned primarily with body image and ability to afford a new prosthesis.[193,212,213 and 214]

### Limb Salvage Surgery

Improvements in survival of patients with osteosarcoma coupled with better imaging techniques, especially MRI, have led most centers to offer patients limb-sparing procedures for lesions about the knee, shoulder, and hip. In view of the close margins used in some limb salvage procedures, higher rates of local recurrence might be anticipated as compared to those associated with amputation, but this has not been the case. Some had feared that patients undergoing limb salvage would have an outcome worse than that in patients treated by amputation. Indeed, investigators of the GPO found the distant failure rate for patients treated by en bloc resection to be significantly higher than that for those undergoing amputation. [172,215] Although no randomized studies have been performed, carefully selected patients treated by experienced surgeons with limb-preserving operations do not appear to have a survival disadvantage. [193,203,216,217 and 218] The role of preoperative chemotherapy in rendering limb salvage surgery safer is unclear, although the administration of chemotherapy before resection seems to render the procedure easier by reducing the edema around the tumor and, on occasion, shrinking the tumor. One center has shown that patients with a less than wide margin and poor histologic response to presurgical chemotherapy have a higher risk of local recurrence than those with a wide margin and a good histological response. [219,220 and 221] However, the local recurrence rate was not high in a large multi-institutional study in which patients did not receive preoperative chemotherapy before either amputation or local resection (MP Link, unpublished data). [122] If the possibility of accomplishing complete excision is in doubt, amputation is the indicated procedure; local recurrence is intolerable,

because metastatic spread of tumor invariably follows.[219,222] The administration of chemotherapy cannot compensate for inadequate surgery.

The longevity of endoprosthetic devices used in limb reconstruction remains problematic, and late infections and graft failure may result in delayed amputations for patients treated by limb resection. Recent reviews suggest that limb-sparing surgery is associated with a higher rate of reoperation for complications, but a better functional outcome is achieved.[193] As young patients with osteosarcoma survive normal life spans, possibly they will outlive their endoprosthetic devices and allograft replacements, and pessimism about the *ultimate* longevity of these replacements may be warranted. However, late mechanical failures likely will be reparable with further surgery.

Screening candidates for limb salvage requires a careful staging workup, including a high-quality MRI of the entire involved bone. The decision to perform a limb-sparing procedure as opposed to amputation is based on the extent of tumor within the medullary cavity, the relationship of the soft tissue mass to the surrounding nerves and blood vessels, the extent of muscle involvement, and invasion of the adjacent joint. In most instances, the joint is not involved. MRI is the most important tool in making this assessment and has replaced the need for angiograms and CT scans.[187,194,195,223]

On the basis of reports from previous studies (discussed in the section Presurgical Chemotherapy), the degree of primary tumor response to presurgical chemotherapy also might be important in deciding whether to proceed with limb-sparing resection. The response to preoperative chemotherapy can be assessed by dynamic MRI, although thallium scans and positron emission tomography scans also have been proposed as accurate modalities.[224,225,226,227,228,229] and [230] Patients presenting with a pathologic fracture may be poor candidates for limb-sparing surgery.[222] Although some investigators have reported healing of pathologic fractures during presurgical chemotherapy, allowing for limb salvage surgery to be undertaken safely,[231] most surgeons recommend amputation rather than limb salvage surgery because of an unacceptably high local recurrence rate when more conservative surgical techniques are used.[222] The definitive answer to the question of whether patients with pathologic fractures should undergo limb salvage surgery is unknown, but clearly such patients are at an increased risk for local recurrence and should be selected for limb salvage with great care. The age of the patient, location of the primary tumor, and functional desires of the patient also are important considerations. A reasonable estimate is that 80% or more of patients with nonmetastatic extremity osteosarcoma are being treated with limb-preserving surgery but, in each case, the decision to perform such a procedure should weigh the risks and benefits as compared to amputation.

Once a tumor is resected, a variety of options are available for reconstruction of an affected limb. In the upper extremity, osteosarcomas most frequently involve the proximal humerus. In most cases, resecting the proximal humerus and some surrounding muscle is possible with or without a portion of the scapula, preserving the neurovascular supply to the arm and hand (the so-called *Tikhoff-Linberg resection*).[232] Options of bony reconstruction include osteoarticular allografts, metallic prostheses, composites of allograft and prosthesis, and vascularized or nonvascularized fibular grafts. If the entire deltoid and rotator cuff are resected, performing a shoulder arthrodesis may be preferable. Even if no reconstruction is performed, preserving the hand is superior to any currently available upper-extremity prosthesis. Tumors of the radius and ulna are rare and, depending on their size and location, can be reconstructed with allografts or autografts (fibula).

In the lower extremity, osteosarcomas are located most frequently in the distal femur, proximal tibia or, less commonly, proximal femur. In these sites, the reconstruction options include osteoarticular allografts, metallic prostheses, arthrodeses (achieved either by allograft or autografts), or allograft-prosthetic composites. Each of these alternatives has advantages and disadvantages, and no carefully controlled studies have documented superiority of one technique as compared to the others. Deciding which specific reconstruction to use is complex and involves a number of factors, but probably the most important is the experience and preference of the surgeon. Finally, some primary tumors can be resected without the need for bone reconstruction. Such sites include the proximal fibula, rib, clavicle, scapula, and parts of the pelvis (Fig. 35-6). Lesions of the distal tibia are best treated by below-knee amputation, and for tumors in the hands and feet, ray amputations usually are possible.

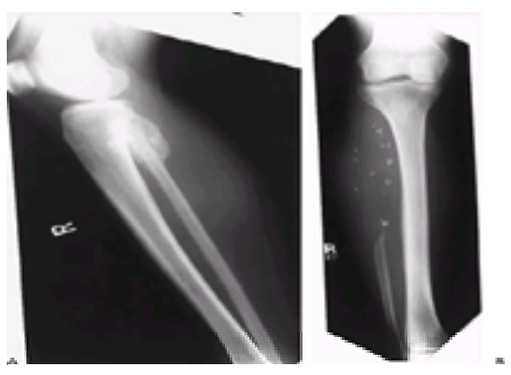


**FIGURE 35-6. A:** Lateral radiograph of an osteosarcoma of the right proximal fibula after presurgical chemotherapy. **B:** Postoperative radiograph demonstrating the surgical defect from resection of the proximal fibula without reconstruction. The peroneal nerve was preserved, although frequently, sacrificing it is necessary. The patient has essentially normal function. If the peroneal nerve is resected, the functional loss is minimal and can be accommodated by an ankle-foot orthosis or a posterior tibial tendon transfer.

The pelvis presents a most challenging site. If the lesion is in the ilium, pubis, or ischium, resection of the tumor with no need for reconstruction may be possible. Lesions involving the acetabulum are more complicated. Although hemipelvectomy (hind-quarter amputations) previously were the only surgical option for such lesions, en bloc excision of the hemipelvis with preservation of the extremity now can be performed in most instances with equal local tumor control. Reconstruction of the resultant defect has been difficult, and metallic endoprostheses have had limited success (80% failure) because of difficulties with the fixation of the prosthesis to the remaining pelvis. The large lever arms and forces applied to the fixation have resulted in frequent loosening and migration of the endoprosthesis. Other methods of obtaining structural integrity have included the use of allografts, fusion of the proximal femur to the ilium or sacrum, attempting a reconstruction with an allograft or a custom metallic prosthesis, and internal hemipelvectomy with no replacement at all (Fig. 35-7).[233,234,235,236,237] and [238] When no replacement is used, a gap between the femur and pelvis is bridged by scar tissue from the adjacent soft tissue, and this fibrous union allows the patient to bear approximately 80% of the body weight.[233] Approximately 2 to 4 inches of limb shortening occurs, which requires additional shoe lifts, and most patients use a cane or crutch. However, even with these limitations, this procedure appears to be a useful alternative to amputative hemipelvectomy, with superior functional results.[233] Whatever reconstruction is chosen, the complication rate is very high as compared to other sites, and achieving negative margins is difficult.[235,237,239,240,241,242,243] and [244]

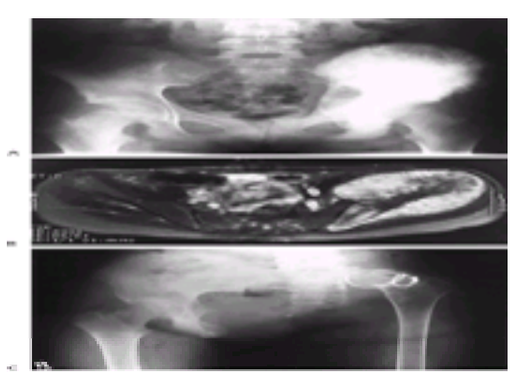


**FIGURE 35-7. A:** Radiograph of an osteosarcoma of the left ilium after presurgical chemotherapy. **B:** Magnetic resonance imaging demonstrates the extent of the lesion throughout the ilium with extension into the iliacus muscle and gluteal muscles. It abuts the sacroiliac joint. **C:** Postoperative radiograph 4 years after internal hemipelvectomy. Because of the extent of the bony and soft tissue resection, no formal reconstruction was carried out. The femoral head was wired provisionally to the sacrum, where it has remained. The patient is able to ambulate with a lift in the shoe and a brace for a partial foot drop.

Osteosarcomas of the spine and sacrum are rare, and treatment must be individualized. Resections are possible at times, but achieving tumor-free margins while retaining neurologic function is very difficult. Multimodality therapy combining limited surgery with irradiation is used frequently. Rarely, osteosarcomas in the spine and sacrum are treated by chemotherapy and irradiation alone.[245,246,247 and 248]

The various alternatives for limb salvage for extremity osteosarcomas are similar in adults and children but, in children, the added factor of remaining growth renders reconstructions more complicated. Specifics of the various reconstruction techniques are detailed here.

### *Osteoarticular Allografts*

Osteoarticular allografting is one alternative for reconstruction of tumor defects of the proximal tibia, distal femur, or proximal humerus.[249,250,251,252,253,254,255,256 and 257] Grafts are procured according to the guidelines of the American Association of Tissue Banks and are stored in a fresh frozen state at –80°C until needed. They are size-matched to specific patients using radiographs of the involved bone and allograft, and they have been shown to be safe from the standpoint of transmission of viral and bacterial disease, owing to the rigor of the procurement protocols.[258,259] Allografts are immunogenic, but the immune response is minimized by fact that they are nonvascularized, and freezing reduces their antigenicity.[260,261,262 and 263] These grafts initially were thought to be resorbed slowly and replaced by host bone but, in practice, host invasion occurs primarily at the allograft-host junction and along the surface of the bone.[264]

Osteoarticular allografts in young people offer certain advantages for reconstruction not available with metallic prostheses.[256] They provide articular surfaces for the involved bone and obviate the need to resect the adjacent articular surface and growth plate. Allografts provide ligaments for joint reconstruction, including cruciate ligaments, and sites for attachment of host tendons (Fig. 35-8, Fig. 35-9). Once the osteosynthesis heals, the longevity of the allograft is anticipated to be superior to that of metallic implants, because they are not subject to loosening, particulate wear debris, and mechanical breakage. If the joint surface deteriorates, the joint can be revised employing a standard total knee prosthesis, and they are not subject to stress shielding and loss of bone stock, as are metallic replacements.

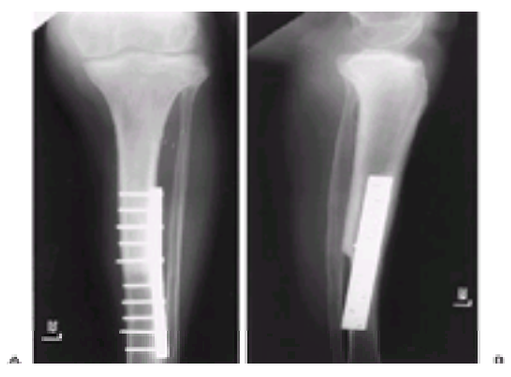


**FIGURE 35-8.** Anteroposterior **(A)** and lateral **(B)** radiographs 5 years after limb-sparing resection of an osteosarcoma of the proximal tibia and reconstruction with osteoarticular replacement of the proximal tibia. The osteosynthesis was achieved with a plate, and the bones have united.

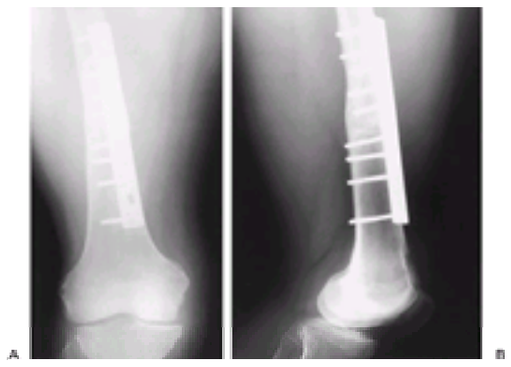


**FIGURE 35-9.** Anteroposterior **(A)** and lateral **(B)** radiographs 4 years after limb-sparing resection of an osteosarcoma of the distal femur and reconstruction with osteoarticular replacement.

Allografts have obvious potential problems and are subject to fracture, nonunion, joint instability, and infection.[256,265,266,267,268,269 and 270] The procedure is technically challenging, and the rehabilitation process is prolonged. Allografts are not meant to allow the patient to return to athletic activities (a drawback in young children), but most limb salvage reconstructions are associated with this limitation.

An MRI after presurgical chemotherapy is used to make the final assessment of resectability. A T1-weighted coronal image is the best study to determine the medullary extent of the lesion and the presence of skip metastases.[194] Sufficient bone must remain after resection of the tumor (usually with 3 to 5 cm of adjacent uninvolved marrow) to achieve osteosynthesis. The size of the patient is a definite issue: It is unlikely that a child younger than 8 to 10 years of age will be large enough to make it possible to find an osteoarticular graft of appropriate size. Practitioners must understand that in girls younger than 12 and in boys younger than 14, other procedures, such as contralateral epiphysiodesis or ipsilateral lengthening, may be necessary at a future time to prevent leg length inequality.[271,272] Usually, gaining 1 to 2 cm of length in older children is possible at the time of the operation by using a graft that is longer than the length of bone resected.

The technical aspects of the procedure are similar to those in adults.[250,256,257,273] A wide, intra-articular resection is performed. If tumor involvement of the joint is a concern, the joint can be inspected through a small arthrotomy at the beginning of the procedure. If the joint is found to be involved, the wound is closed and an extra-articular resection or amputation can be performed. Otherwise, a longitudinal incision is made anteromedially or anterolaterally, and a cuff of uninvolved muscle is retained around the tumor. The neurovascular structures are identified and preserved, and the bone is divided with an oscillating saw 3 to 5 cm above the level of involvement predicted by the MRI. The joint ligaments and cruciate ligaments are divided, leaving as much length as possible on the remaining host bone without violating the tumor. The specimen is examined by an experienced pathologist to assess the margins of resection. An osteoarticular allograft then is thawed, cultured for aerobic and anaerobic organisms, and cut to fit the length of the defect. If the patient is skeletally immature, the allograft bone is fashioned 1 to 2 cm longer than the length of bone resected, depending on the integrity of the soft tissues. The osteosynthesis can be achieved with broad DC plates or intramedullary rods. The former appear to achieve osteosynthesis more reliably because of more rigid fixation, whereas the latter are associated with a lower fracture rate.[274,275] Cruciate ligaments are primarily repaired using heavy, nonresorbable sutures, and then the capsular structures are repaired primarily adjusting for appropriate length. Host menisci are preserved unless resected for tumor considerations. The patellar ligament is repaired in a pants-over-vest fashion to the graft ligament in proximal tibial lesions. The joint should be stable at the completion of the repair. The soft tissues are closed with the aim of achieving a complete muscle closure over the graft. Depending on the extent of muscle resection, this closing may require local rotational (gastrocnemius) flaps or, at times, free vascularized transfers.

Osteosarcomas of the proximal humerus can also be resected with wide margins preserving the vasculature and nerves to the upper extremity (Fig. 35-10). The deltoid muscle (and frequently parts of the rotator cuff) are resected, and the axillary nerve and glenoid may have to be sacrificed. The biologic reconstruction options include an osteoarticular allograft or an allograft-prosthetic composite or a shoulder arthrodesis.[255,270,276,277,278,279,280,281 and 282] Even leaving the shoulder flail offers function superior to that of an amputation. The complication rate with osteoarticular allografts is high,[255,270,277] leading some surgeons to prefer allograft-prosthetic composites to reduce the incidence of fracture.[277,283,284] The overall function is good in approximately 70% of patients, but abduction above the horizon seldom is achieved. An arthrodesis allows more powerful abduction and may be appropriate in people interested in manual occupations, but achieving an arthrodesis is difficult. Combinations of allografts and vascularized fibulae increase the likelihood of a successful arthrodesis. Motion—especially rotation—is sacrificed for power of

abduction.[249,256,277,278,285]

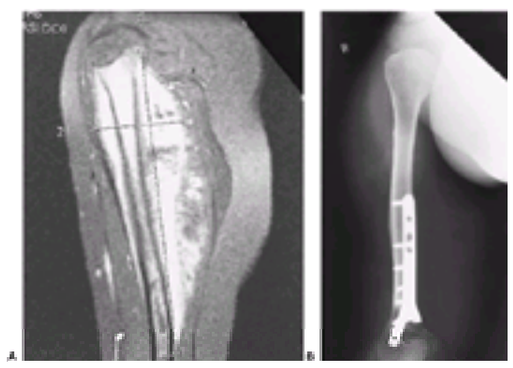


**FIGURE 35-10. A:** Magnetic resonance imaging of a 12-year-old with osteosarcoma of the proximal humerus. The soft tissue extent of the mass is extensive, and it involves the majority of the humeral shaft. **B:** Radiograph 1 year after limb-sparing resection and reconstruction with an osteoarticular allograft. Reasonable range of motion of the shoulder and elbow is present.

Postoperatively, the extremity is immobilized in a cast or brace for 6 to 8 weeks, and motion is begun in a protective brace at that time. In the lower extremity, weight bearing is restricted to touchdown status but, when early signs of bony union are visible, partial weight bearing in a brace can begin. Osteosynthesis in patients receiving chemotherapy usually occurs between 9 and 12 months.[257] The early complications include wound healing problems that are less frequent with experience of the surgeon and the liberal use of rotational and free flaps and skin grafts. [250,252,256,265,267,268] and [269] When wound dehiscence occurs, it should be treated aggressively with débridement, closure, and soft tissue transfers, if necessary. Nonunion occurs in 10% to 25% of allografts in children, and the incidence appears to be greater in patients receiving chemotherapy.[250,256,257] This complication usually is managed easily by revision of the fixation and autogenous bone grafting. Fracture usually is a late complication, occurring between 3 and 8 years from the procedure. The incidence is approximately 20% of allografts in children. [257] Fractures can be treated by standard management techniques but, on occasion, a fracture will result in failure of the implant and will require removal of the graft and reconstruction with a second graft, a metallic prosthesis, or amputation. Also possible may be salvaging the graft by augmenting it with a vascularized fibular graft (either as a free transfer or a pedicle graft for tibial sites) if the joint is functional. [286,287,288] and [289]

Infection occurs in 10% to 15% of pediatric allografts [249,252,256,266,267,270] and is a more devastating complication. A deep infection almost always results in failure of the graft.[266] In perhaps one-half of such cases, removal of the graft, insertion of an antibiotic-impregnated polymethyl methacrylate cement spacer, and intravenous antibiotics followed by a second allograft in 3 months will achieve a successful result. [290]

The functional results are satisfactory in approximately 60% to 70% of children with high-grade sarcomas requiring adjuvant chemotherapy. [250,252,255,270,291,292] Running, jumping, and contact sports are not encouraged but sometimes are performed by these young patients. For growing children, attention must be paid to limb length. [272] For children nearing skeletal maturity (the majority of patients), this is seldom a problem, because the limb can be lengthened 1 to 2 cm at the time of the reconstruction. If further growth of the contralateral side leads to an ultimate discrepancy of 2 cm or less, no correction is necessary. For girls in the 10- to 12-year age group or boys in the 12- to 14-year age group, a contralateral epiphysiodesis may be necessary. An alternative is to delay equalization until after skeletal maturity; if the discrepancy is significant (5 cm or more), consideration is given to closed femoral shortening of the uninvolved side or limb lengthening of the involved side.

Children younger than 8 years seldom are candidates for an osteoarticular allograft about the knee. An amputation, rotationplasty, or the use of an “expandable” prosthesis is preferable, although a prosthesis is less likely to be successful in children younger than 8 years.

### *Intercalary Allografts*

In some instances, an osteosarcoma is located in the diaphysis of the bone, such that the adjacent joints and metaphyses can be spared. In young patients in whom a margin of bone can be obtained while preserving the proximal and distal femoral metaphyses, an intercalary allograft can be employed to reconstruct the diaphysis.[249,250,277,293,294] Fixation can be achieved with plates so that the growth plate can be spared or, if the epiphysis is needed for fixation, the fixation devices (screws) in the epiphysis can be removed to allow growth once the osteosynthesis has healed. Some surgeons employ vascularized fibular grafts with or without allografts to achieve a similar reconstruction. [286,287,288] and [289] Good function has resulted from intercalary allografts alone. [249,293]

Some lesions of the distal diaphysis of the femur extend close to the growth plate but not into the epiphysis. Such a situation may afford the possibility of preserving the epiphysis but not the growth plate and still achieving a negative bony margin. To do so requires careful analysis of a good-quality MRI with a radiologist who has expertise in judging tumor extent. If the epiphysis can be spared, doing so seems preferable, so that an affected child's own joint surface can be preserved. This provides a durable reconstruction and excellent function if sufficient quadriceps function can be preserved. Limb length equality may have to be achieved by other means if such a child is very young. A similar situation may occur (but less commonly) in the proximal tibia.

### *Metallic Prostheses*

Metallic prostheses have been employed successfully to reconstruct tumor defects in adults, and similar approaches have been applied to children.[295,296,297,298,299,300,301,302] and [303] These devices are manufactured as casts of cobalt, chrome, or steel, or they are machined from titanium. The advantages of endoprostheses are the immediate stability allowing weight bearing, freedom from postoperative immobilization, and a low incidence of early complications. The infection rate has been reported to be between 0% and 35% and varies with the site of the reconstruction. [304,305,306,307,308] and [309] Fatigue fracture of the metal and loosening of the prosthesis are other potential problems. Fatigue fracture of the metal is a design and stress problem that potentially can be improved by changes in casting methods, use of stronger metals, or improvements in stress-relieving joint designs. Loosening of the endoprosthesis at the cemented bone-prosthesis interface is a problem that is not resolved as easily, but it is minimized by the use of meticulous cement technique and stress-reducing total joint mechanics, such as a rotating-hinge knee design. Recent design modifications that allow for bony ingrowth at the stem-host junction and uncemented designs also may prove useful.[305,310,311] These improvements encourage extramedullary ingrowth of the host's own bone along the metallic endoprosthesis shaft, thereby providing a biologic fixation of the prosthesis.

As with allografts, limb length is an issue, especially because the adjacent proximal tibial or distal femoral growth plate may have to be either resected or crossed by the implant to accommodate the prosthetic knee joint. In one series,[312] the operative extremity limb length was 79% that of the normal limb after treatment. Also, the tibial growth plate has been demonstrated to continue to grow after cementing a stemmed prosthesis across it. [313]

Several different types of “expandable” prosthesis that have been devised can be lengthened as the child grows. [291,295,297,298,302,304,314,315,316,317] and [318] These constructs provide immediate stability and early return to function but present the caveat of poor longevity in young active children. Problems include fixation and stress shielding in the growing child, mechanical failure of these more complex implants, and debate regarding the optimal fixation to the host bone. All these concerns are shared with prostheses in adults but, in the child, issues include a longer life span (if the tumor treatment is successful) and greater activity than that in adults. A “growing prosthesis” implanted in children almost certainly will loosen or mechanically fail with time or simply may be too small when such children reach adulthood and so may need to be replaced with another prosthesis.[314] Several centers have reported a steadily declining survival curve for metallic prostheses in children. [302,304,314,316] Most often, these prostheses can be revised to another prosthesis. The need for future revisions of the prosthesis if an affected child survives an entire life span should be expected. One concern is that over time, the bone stock remaining will render the revision of a prosthesis difficult, if not impossible.

Metallic prostheses are used most commonly for lesions about the knee and employ rotating- or pure-hinge knee implants. Prostheses for the proximal femur are similar to those in adults, and growth is not as much an issue in this location. The indications are similar to those for allografts (a resectable lesion in a child older

than 8 years), although limbs in patients requiring extra-articular resections can be reconstructed by prostheses as well. The technique of resection is similar to that for an allograft. Achieving a wide resection and preserving sufficient muscles, nerves, and vessels are desirable. Careful preoperative planning is necessary to ensure that a suitable implant is available. Several types of prostheses designed for children have a mechanism for serial lengthening of the implant. These prostheses are complex, and their mechanical failure rate is fairly high. Recent design features have improved the ability to expand the prostheses as the child grows. In most cases, the prosthesis will have to be revised to a standard prosthesis after skeletal maturity.

Recently, modular tumor prostheses have been available in North America and Europe [299,308,309,319,320] but, in smaller children, a custom implant may be necessary. The development of modular prosthetic systems for segmental bone and joint replacement after tumor resection increases the options for surgeons in planning tumor resections (Fig. 35-11). Such prostheses are available for the distal and proximal femur, proximal tibia, and proximal humerus. Each consists of components appropriate for the segment to be replaced (usually an intramedullary stem, a body segment, and a joint component), and each component comes in various segmental lengths and stem dimensions. Such modular systems permit almost immediate availability and choice of sizes suitable for most tumor sites for most patients and for reconstructions of almost any length, so that individualized, customized devices need not be ordered for each patient. The advantages of modular prostheses include the simpler design, ready availability, flexibility with regard to the length of resection at the time of operation, and lesser cost than that of custom prostheses. For distal femoral lesions, the length of the implant is constructed by combining various-length body segments with the distal femoral component to match the resected length. The articular surface of the proximal tibia is resected (preserving the physis), and a smooth-stemmed tibial component is employed using cement on the tibial surface and in the medullary canal. Studies have indicated that the growth plate has sufficient strength to break the cement mantle over time and allow for growth of the proximal tibia.[313] Alternatively, an uncemented smooth-stemmed tibial component can be employed and revised after skeletal maturity. Lengthening is accomplished by replacing the body segment of the prosthesis by a new segment that is longer (usually 1 to 2 cm per lengthening) than the initial one. This requires dislocation of the knee and a special device to loosen the Morse tapers. Lengthening can be a difficult procedure, because an unforgiving fibrous capsule develops around the prosthesis. In most instances, it must be resected completely to gain length. Nerves and vessels embedded in scar must be protected carefully. Young children require two to three lengthening procedures to compensate for growth discrepancies of 1 to 9 cm. There are limited data on the number of children in the 8- to 10-year age group who have reconstructions with expandable prostheses around the knee and reach skeletal maturity with equal limb lengths.

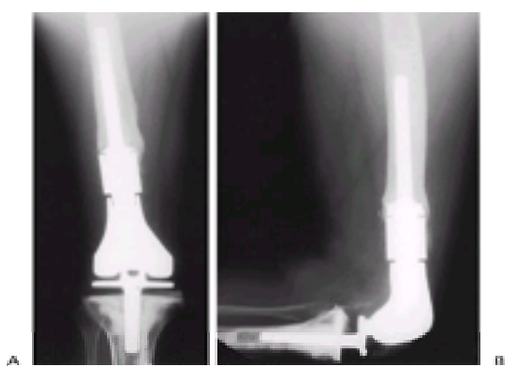


**FIGURE 35-11.** Anteroposterior **(A)** and lateral **(B)** radiographs of an osteosarcoma of the distal femur after resection and reconstruction with a commonly used modular, rotating-hinge prosthesis. The precise length of this prosthesis can be designed at the time of operation by combining segments of different lengths. The rotating-hinge knee joint compensates for ligamentous loss.

Prosthetic reconstruction of the proximal tibia is complicated by the need to reattach the patellar tendon to the prosthesis to gain active knee extension. Much research has gone into developing tissue ingrowth into the prosthesis to achieve this extension, but most surgeons rely on synthetic materials to attach the tendon to a loop or pad on the prosthesis and augment this with a medial gastrocnemius flap to gain tissue to which to attach the tendon. This approach seems to be moderately successful in restoring the extensor mechanism.

The complications of endoprostheses include metallic wear debris in the soft tissues (100% of titanium implants), expansion failures (approximately 30%), loosening of the prosthesis, and mechanical failure.[298] The reported infection rates vary with the series but appear to be much higher in the tibia than in the femur.[304] Careful soft tissue closure and use of gastrocnemius rotation flaps (when necessary) are critical to reducing the infection rate. Complications of lengthening include dislocation, joint contracture, and nerve palsies. The 5-year revision-free survival of the prosthesis in one series was 15% for an expandable prosthesis and 72% for the modular prosthesis.[298] Reported results of recent series of children with osteosarcoma treated with endoprosthetic replacement are encouraging. The local recurrence rate is acceptably low (5% to 11%), but the complication rate remains high (55% to 80%).[216,297,304,314,315,321] Mechanical failure or loosening is almost inevitable in young children receiving these prostheses, but usually such prostheses can be revised when the children reach skeletal maturity.

Similar prostheses are available for use after resection of proximal humerus osteosarcomas. They work well for restoring bony integrity, but achieving muscle attachments to the prosthesis is difficult. Heavy suture or arterial graft materials have been used to accomplish this with varying success. Stability of the shoulder joint may be a problem, and affected patients likely will not achieve good active shoulder mobility.

### *Allograft-Prosthetic Composites*

Another alternative to limb reconstruction is to combine a standard knee or proximal humerus prosthesis with an allograft for lesions about the knee or shoulder.[250,256,277,283,284,291,322,323,324,325] and [326] This combination offers the advantage of joint reconstruction employing a more standard arthroplasty and of restoring bone stock with allograft bone. In the shoulder, the composites offer the advantages of protecting the allograft with a metallic stem and avoiding periarticular fractures seen in osteoarticular grafts. In addition, the allograft provides attachment sites for the rotator cuff and other muscles. Similarly, in the proximal tibia, the allograft provides a site for attachment of the patellar tendon, and the prosthesis provides a stable joint reconstruction. At all sites, the composites allow for modularity and, in theory, may provide a reconstruction more durable than that of osteoarticular allografts or metallic prostheses.

### *Arthrodesis*

Although arthrodesis was a popular reconstruction in the early days of limb salvage ( Fig. 35-12), especially about the knee, the availability of mobile joint reconstructions has largely replaced this as an option. In the knee, the major advantage is durability. Once the construct heals, the long-term outcome is good and provides a reconstruction that can be used safely in some sports and heavy labor.[249,278,281,327,328] The major disadvantage is the inability to flex the knee, which is problematic while sitting in tight spaces, such as theaters and airplanes.

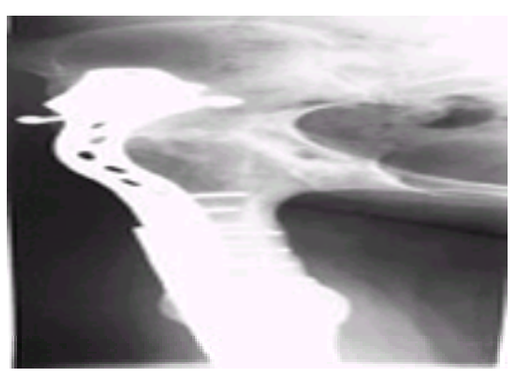


**FIGURE 35-12.** Radiograph 10 years after extra-articular resection of an osteosarcoma of the femoral neck, with a pathologic fracture and contamination of the hip joint. Reconstruction was accomplished with an intercalary allograft to create a hip arthrodesis. The patient walks with a nearly normal gait and can sit without

difficulty because of the flexibility of the lumbar spine.

In the shoulder, it is perhaps a more acceptable option. It allows for powerful positioning of the shoulder and arm in space and is useful in an athletic person or a heavy laborer. The benefit of power to abduction and forward flexion is at the cost of limitation of rotation of the shoulder. Achieving an arthrodesis of the shoulder is difficult after resection of a tumor, and combinations of allografts and vascularized or nonvascularized fibular grafts and special fixation devices are necessary to achieve solid union.

***Rotationplasty***

Young children with high-grade sarcomas of the knee area have limited options for reconstruction after resection of the tumor. Limb-sparing procedures have the drawbacks of activity restrictions, high complication rate, limb length inequality, and complexity. An above-knee amputation for a distal femoral osteosarcoma in very young patients leaves such children with a very short lever arm to power a prosthesis, and it becomes relatively shorter as the child grows (i.e., the child's opposite limb grows, but the femoral stump does not).

The operation described by Borggreve and adapted for congenital defects (e.g., proximal femoral focal deficiency) by Van Nes has been applied to the tumor setting by Salzer and provides a reconstruction option for certain situations. [329,330,331,332,333,334,335,336,337,338,339] and [340] It can be thought of as an intercalary amputation of the distal femur (or proximal tibia). The reconstruction employs the distal leg that is rotated 160 to 180 degrees, and this provides the advantage of a longer lever arm and an active “knee” joint provided by the ankle and foot ( Fig. 35-13).

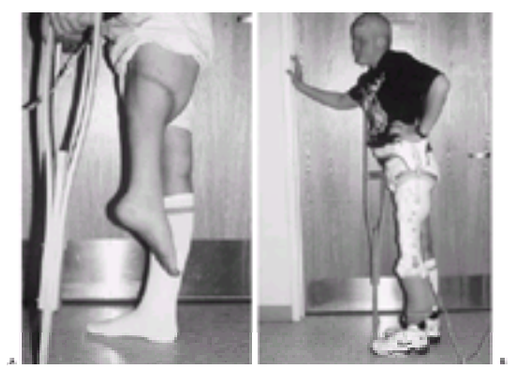


**FIGURE 35-13. A:** Rotationplasty performed for a distal femur osteosarcoma with foot inverted and ankle functioning as a knee. **B:** With the below-the-knee external prosthesis in place, the patient has excellent lower-extremity function. (See Figure 45-3 and Figure 45-4.)

The indications for rotationplasty include a distal femoral or a proximal tibial osteosarcoma in skeletally immature patients or in those who want to continue sporting activities, or as a salvage procedure for a failed distal femoral reconstruction. It must be possible to preserve the sciatic nerve and its branches, although the vessels may be divided and anastomosed to increase the margin if necessary. The advantages are the wide margin that includes the skin, adjacent knee joint, and thigh muscles; the avoidance of phantom pain; rapid healing of the osteosynthesis site; and a relatively low complication rate. The obvious drawback is the appearance, which is repulsive to some. At times, the patient may be more in favor of this option than are the parents or the physicians. Interestingly, young children usually do not view the procedure as an amputation because the foot remains and, with a good prosthesis, they are able to function better than other amputees. [341,342,343] and [344] Follow-up studies have not demonstrated any adverse psychological outcomes, [330,334,345,346] and carefully selected patients who have undergone the procedure usually are content.[335] Preoperative discussions must be honest and complete, so that both patient and family are aware of the nature of the procedure and the expected outcome. A helpful approach is for them to meet with a physical therapist who is familiar with this procedure, to view videotapes of patients who have undergone the procedure and, ideally, to meet a patient with a rotationplasty. All these techniques are necessary in addition to considerable time spent explaining the rationale and the relative advantages and disadvantages of this and the other options, such as amputation and limb-sparing procedures. Recently, the number of patients willing to undergo this procedure has diminished; many prefer to try a limb-sparing procedure and to reserve rotationplasty until or unless limb sparing fails.

The procedure itself is well described in the literature. The tendency is to make the thigh long, so that the rotated “knee” appears to be distal to the contralateral knee. Unless the femoral resection is fairly high, the rotationplasty knee will be distal to the contralateral normal knee. Accurately predicting remaining growth is difficult because the distal tibial physis and the tarsal bones become analogous to the contralateral distal femoral growth plate.

In general, a boy older than 14 and a girl older than 12 probably should have the rotationplasty knee placed opposite the contralateral knee. For younger patients, placing it 2 to 4 cm more caudal is appropriate. [335,347] The vessels may be resected with the specimen to increase the amount of normal tissue margin. An anastomosis of the vein and artery can be completed after achieving osteosynthesis. Flushing the leg with a perfusate of heparin and cool Ringer's lactate solution is helpful in decreasing the effects of the ischemia time, which should be less than 1 hour. At times, the small vessels will spasm and necessitate revision of the anastomosis, but this does not lead to loss of the leg or a compartment syndrome. An alternative is to dissect the vessels free from the tumor and loop them carefully back on themselves with the nerves. Postoperatively, the wound is observed for healing and perfusion of the foot. The patient is placed in a spica cast or brace for 6 weeks to allow early healing of the osteosynthesis site and to allow mobilization. A prosthesis can be provided at 6 to 8 weeks, and partial weight bearing can be continued until the osteosynthesis site has healed. Stressing range of motion of the ankle (especially full plantar flexion) is important early in the postoperative course; if a patient loses motion, the ankle should be splinted in full plantar extension while at rest. [335]

This procedure has been described also for tumors of the proximal tibia, with successful results. [345,348] Modifications of this procedure have been described for lesions about the hip or those involving a large portion of the proximal femur. [216,349,350] The ilium and distal femur must be preserved for this procedure to provide a “hip” and a “knee.” A similar procedure has been reported for lesions about the shoulder. [351]

A rotationplasty offers a durable and functional, albeit cosmetically unpleasant, reconstruction option for selected patients with a sarcoma. For very young patients with a distal femoral or proximal tibial lesion, it avoids the repeated surgical procedures necessary to achieve limb length equality and allows affected children to run and play exceedingly well. The other useful indications include a failed limb salvage procedure or a pathologic fracture when amputation would be the only alternative.

**Radiotherapy in the Management of the Primary Tumor**

In the past, radiotherapy was recommended to treat the primary tumor in osteosarcoma. However, osteosarcoma has been found to be a highly radioresistant lesion. Radiation doses of less than 6,000 cGy have been associated with only transient tumor control, [352] and viable tumor has been observed in amputation specimens after doses of 8,000 cGy or more.[353,354] The failure of conventional radiotherapeutic fractionation techniques to provide durable local control of osteosarcoma may be explained by the presence of a relatively high percentage of hypoxic tumor cells and *in vitro* evidence that osteosarcoma cells may have increased capacity to repair sublethal radiation injury.[355]

Before the 1970s, when osteosarcoma was almost uniformly fatal, a strategy of primary radiotherapy followed by delayed amputation evolved in an effort to avoid mutilating surgery in patients destined to die of their disease. [1,353] High-dose radiotherapy was administered initially, and ablative surgery was used only for those patients remaining free of disease 4 to 6 months after the completion of radiotherapy. However, in a subsequent assessment, primary radiotherapy was found to result in few responses; local recurrences appeared in virtually all cases, and palliation (the ostensible goal of delayed surgery) was poor. [352]

With improvements in the control of micrometastatic disease by systemic chemotherapy, the majority of patients should now be approached with curative intent, so

local recurrence is intolerable. Apparently, the overall control rate of the primary tumor with radiotherapy is significantly less than with ablative surgery, so radiotherapy has little role in the management of primary osteosarcomas that are controllable by surgery. The use of hypofractionated radiotherapy or other novel radiation fractionation techniques, with or without radiosensitizers to overcome the capacity of osteosarcoma cells to repair sublethal damage, may result in more durable control of primary osteosarcoma, although the increased soft tissue injury that results may limit this approach. [355]

The increased normal tissue injury associated with large dose-per-fraction radiation argues for multiple small fractions (hyperfractionation) in an effort to spare normal tissues. Because of the substantial risk of local recurrence in osteosarcomas treated primarily with irradiation, primary radiotherapy or radiotherapy as an adjunct to debulking surgery should be reserved for patients with unresectable tumors and patients with axial skeleton tumors that cannot be excised completely. Such patients have fared poorly in the past because durable local control has been difficult to achieve.

## Adjuvant Treatment

### *Chemotherapy*

Although control of the primary tumor is accomplished reliably by surgery, data from historical studies and recent controlled trials indicate that more than 80% of patients with osteosarcoma treated with surgery only will develop metastatic disease. Microscopic, subclinical metastatic disease is thus present at the time of diagnosis in the majority of patients without overt metastases. Evidence from animal models supports the notion that subclinical metastases can be eradicated by chemotherapy if the treatment is initiated when the total burden of metastatic tumor is sufficiently low [356,357 and 358] and provides the rationale for adjuvant chemotherapy in osteosarcoma. However, osteosarcoma is a notoriously drug-resistant neoplasm, and the list of drugs active against macroscopic disease and thus applicable in the adjuvant setting is disappointingly short. Before 1970, none of the drugs tested had produced responses in more than 15% of patients, and most of the responses in phase II studies were partial rather than complete. More promising results were observed in the 1970s and early 1980s in phase II trials of doxorubicin, [359] high-dose methotrexate (HDMTX) with leucovorin rescue,[360,361 and 362] cisplatin,[363,364,365 and 366] and ifosfamide.[367] The hopeless prognosis of patients with osteosarcoma in the 1970s led to the enthusiastic application of these agents singly or in combination as adjuvant therapy after amputation or resection of the primary tumor. An apparent improvement in outcome as compared to the historical experience was demonstrated in a number of uncontrolled trials of adjuvant therapy conducted in the 1970s and early 1980s. Two randomized, controlled trials conducted in the mid-1980s confirmed both the validity of the historical experience as a control for these studies and the favorable impact of adjuvant chemotherapy in the treatment of osteosarcoma. [121,122 and 123]

Reported results of some of the important adjuvant chemotherapy trials of the 1970s and 1980s are summarized in Table 35-2. Concerns have been raised that the favorable results achieved in patients treated with adjuvant chemotherapy might not be sustained with longer follow-up, as late relapses occur, and that adjuvant chemotherapy for osteosarcoma might delay but not prevent relapse. However, results of many of the adjuvant studies reported in Table 35-2 (some with follow-up beyond 20 years) suggest that life tables for relapse-free survival have stable plateaus beyond 4 years and that relapses after 3 years are infrequent. The majority of patients surviving 3 years without evidence of recurrence thus are likely to be cured.

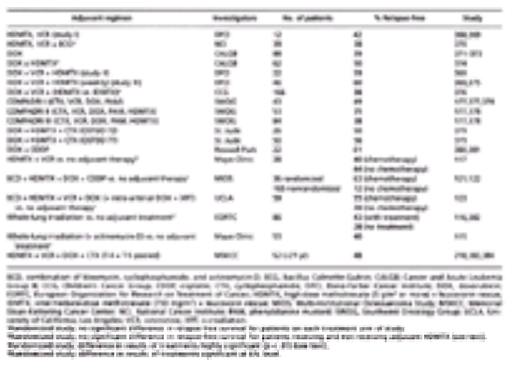


**TABLE 35-2. REPORTED RESULTS OF REPRESENTATIVE TRIALS OF ADJUVANT THERAPY FOR OSTEOSARCOMA**

Examination of the results of adjuvant trials reveals a trend in the direction of improved outcome for patients treated on more recent protocols. Considering that so few drugs have demonstrable activity against macroscopic osteosarcoma, the results reported in adjuvant trials are remarkable. With currently available regimens, approximately 60% to 70% of patients with nonmetastatic osteosarcoma of the extremity will survive without evidence of recurrence. The development of adjuvant regimens has been largely empirical, and newer, more intensive regimens have resulted in further improvements in outcome. The majority of regimens currently in use incorporate doxorubicin, cisplatin, and HDMTX. The role of HDMTX in the treatment of osteosarcoma has been questioned, [385] because a randomized study conducted by the Children's Cancer Study Group[376] comparing HDMTX and intermediate-dose methotrexate in combination with doxorubicin as adjuvant therapy failed to show any benefit for patients receiving HDMTX. The overall outcome was not better than that achieved in studies using doxorubicin alone. In contrast, a more recent study from the Instituto Rizzoli[386] demonstrated superior responses in the primary tumor and a superior outcome for patients receiving HDMTX as compared to those receiving intermediate-dose methotrexate in the context of a multi-agent chemotherapeutic regimen. Other trials also have concluded that serum methotrexate levels achieved in patients may correlate with tumor responses observed and with patient outcome.[387,388 and 389] A peak methotrexate level of 1,000 μM appears to be a "threshold" that must be exceeded to observe a therapeutic effect in osteosarcoma. [387,388]

A trial conducted by the European Osteosarcoma Intergroup compared the combination of doxorubicin and cisplatin alone or doxorubicin and cisplatin alternating with HDMTX as pre- and postsurgical chemotherapy for patients with osteosarcoma.[390] The disease-free survival for patients receiving the two-drug regimen (without HDMTX) was significantly superior to that of patients treated with all three drugs. However, in this study, the intensity of administration of HDMTX was compromised by the design of the study, and the overall outcome of patients in this report is inferior to that observed in other recent studies. Thus, the role of HDMTX in chemotherapy of osteosarcoma requires further study.

The combination of bleomycin, cyclophosphamide, and actinomycin-D[391] was pioneered in early trials from Memorial Sloan-Kettering Cancer Center (Memorial Hospital) and was incorporated into trials conducted by other investigators as well. A subsequent phase II trial at the St. Jude Children's Research Hospital failed to show any benefit,[392] and the regimen of bleomycin, cyclophosphamide, and actinomycin-D no longer is recommended for the treatment of osteosarcoma.

The activity of ifosfamide was demonstrated more recently, and this promising agent is included in some newer regimens currently under study. The Children's Cancer Group (CCG) and the Pediatric Oncology Group (POG) have completed a randomized trial to evaluate the addition of ifosfamide to the treatment of osteosarcoma. Patients were randomly assigned to treatment with cisplatin, doxorubicin, and HDMTX, with or without the addition of ifosfamide. The preliminary results of this trial did not demonstrate a benefit for the patients who were treated with the addition of ifosfamide. [457]

### *Whole-Lung Irradiation*

Prophylactic irradiation of the lungs as an adjuvant to surgical treatment of the primary tumor was evaluated in several trials. Osteosarcoma is not considered to be a radioresponsive tumor; these trials were justified by theoretic estimates of tumor burden in pulmonary micrometastases in patients with subclinical disease and radiobiologic considerations of the "curability" by radiotherapy of patients presenting with fewer than $10^5$ tumor cells in micrometastases.[116,382] Results have demonstrated only marginal (if any) benefit for irradiated patients, [115,116,382] and adjuvant whole-lung irradiation is not incorporated into modern treatment regimens for osteosarcoma.

### *Presurgical Chemotherapy*

The administration of chemotherapy before definitive surgery of the primary tumor (in addition to postoperative adjuvant chemotherapy) has been used with increasing frequency in recent years. The strategy initially evolved from early attempts at limb salvage surgery at the Memorial Sloan-Kettering Cancer Center, where customized

endoprosthetic devices were used for limb reconstruction. As fabrication of these devices required 2 to 3 months, patients were treated with chemotherapy after biopsy while awaiting definitive surgery.[218] Tumor shrinkage in response to chemotherapy facilitated limb salvage, and patients treated with presurgical chemotherapy appeared to fare better than did concurrent patients treated with immediate surgery and postoperative adjuvant therapy. Moreover, histologic evaluation of the excised tumor after treatment with chemotherapy was found to be a powerful prognostic factor for tumor recurrence; patients demonstrating extensive residual viable tumor cells were more likely to develop distant metastases despite continuation of chemotherapy adjuvantly after surgery.[393] The prognostic value of residual viable tumor after presurgical chemotherapy has been confirmed in several trials.[168,172,218,383,384,386,390,394,395 and 396]

To provide uniformity in the assessment of residual viable tumor after chemotherapy, several systems for grading the effect of presurgical chemotherapy have been proposed on the basis of the histologic assessment of cellularity and necrosis in the excised specimen. The grading system developed at the Memorial Hospital by Huvos[393] has been used widely and has served as a model for other systems for grading residual viable tumor ( Table 35-3).[394,397,398] In the Huvos system, complete or near-complete absence of residual viable tumor (grade III or grade IV effect, less than 10% residual viable tumor) is associated with a better prognosis. Lesser effect (grade I or grade II) with more than 10% residual viable tumor is associated with an inferior prognosis. Such grading systems are necessarily imprecise and subject to sampling errors. However, with meticulous attention to adequate sectioning from multiple sites of the surgical specimen, the effect of chemotherapy and an estimate of residual viable tumor can be assessed reliably. In most studies, patients with favorable chemotherapy effect (grade III or grade IV with less than 10% residual viable tumor) fare extremely well, whereas those with more than 10% residual viable tumor after presurgical chemotherapy (grade I or grade II effect) are likely to develop distant metastases. Thus, patients at high risk for the development of recurrent disease apparently can be identified early in treatment on the basis of the poor histologic response of the primary tumor to presurgical chemotherapy. Noteworthy is that in all studies, patients with grade I and grade II chemotherapy effect and residual viable tumor at the time of resection of the primary tumor still have outcomes significantly superior to that of patients who do not receive adjuvant chemotherapy. Thus, patients with grade I and grade II chemotherapy effect and residual viable tumor after initial chemotherapy still can be expected to benefit from the administration of chemotherapy.

| Grade | Effect |
|---|---|
| I | Little or no effect identified |
| II | Areas of acellular tumor osteoid, necrotic, or fibrotic material attributable to the effect of chemotherapy, with other areas of histologically viable tumor |
| III | Predominant areas of acellular tumor osteoid, necrotic, or fibrotic material attributable to the effect of chemotherapy, with only scattered foci of histologically viable tumor cells identified |
| IV | No histologic evidence of viable tumor identified within the entire specimen |

From G Rosen et al. Primary osteogenic sarcoma: eight year experience with adjuvant chemotherapy. J Cancer Res Clin Oncol 1983;106 [Suppl]:55–67.

**TABLE 35-3. HISTOLOGIC GRADING OF THE EFFECT OF PREOPERATIVE CHEMOTHERAPY ON PRIMARY OSTEOSARCOMA**

Although the predictive value of histologic assessment after presurgical chemotherapy is now indisputable, a number of problems have surfaced in the application of grading chemotherapy effect to patient management. Unfortunately, different criteria for the definition of favorable and unfavorable chemotherapy effect are used in different grading systems, rendering comparisons among studies difficult. Especially noteworthy is that in a recent update of the Memorial Hospital experience with presurgical chemotherapy, the implications of “favorable” and “unfavorable” chemotherapy effect have changed somewhat. Apparently, grade IV chemotherapy effect is predictive of favorable outcome. However, the outcome of patients with grade III chemotherapy effect is not significantly superior to that for patients with grade II chemotherapy effect.[171] Thus, only patients who experience a grade IV chemotherapy effect after presurgical chemotherapy might be considered in the most favorable prognostic group.

Perhaps most problematic are the differences among studies conducted to date in the timing of surgery relative to the initiation of chemotherapy, and especially the variable duration of exposure to chemotherapy prior to definitive surgery and histologic evaluation of chemotherapy effect in the primary tumor. Apparently, presurgical chemotherapy regimens of longer duration are associated with a higher proportion of patients achieving favorable chemotherapy effect.[171,399] However, as the duration of presurgical chemotherapy increases, the predictive value of chemotherapy effect for outcome may be lost; longer, more intensive presurgical regimens may produce a greater proportion of patients achieving favorable chemotherapy effect, but the favorable effect obtained from such regimens may not translate into more favorable outcome. An analysis from the Memorial Hospital suggests that this is indeed the case.[171] Thus, as the proportion of patients achieving a favorable chemotherapy effect increases with the administration of longer, more intensive presurgical regimens, the value of this prognostic factor may be lost.

In addition to the value of identifying responsive and nonresponsive tumors, the strategy of presurgical chemotherapy is attractive because of other theoretic considerations.[383] Because chemotherapy is administered very soon after biopsy and diagnosis, treatment of micrometastases known to be present in the majority of patients can be instituted early. This represents a significant advantage over the traditional adjuvant approach, wherein the administration of systemic chemotherapy is delayed by a month (approximately one tumor doubling time for osteosarcoma) or more by surgery and the time necessary for wound healing. Such a delay is likely to be most critical early in treatment, when the burden of micrometastatic disease is high. Earlier treatment might reduce the chance of spontaneous emergence of drug-resistant clones of tumor cells in the micrometastases.[400,401]

One of the most compelling rationales for presurgical chemotherapy is its use as an *in vivo* drug trial to determine the drug sensitivity of an individual tumor and to customize postoperative chemotherapy. Results of studies from the Memorial Hospital and elsewhere suggest that patients whose tumors demonstrate near-complete absence of viable tumor cells in the resection specimen after presurgical therapy are destined to do well when the same therapy is continued postoperatively. Patients whose tumors demonstrate 10% or more residual viable tumor cells after the presurgical regimen have a much less favorable outlook and might benefit from a change in chemotherapeutic agents. Although this is an attractive hypothesis, several objections can be raised on theoretic grounds. Considerations of cell kinetics predict that responsiveness (or lack thereof) of a bulky tumor may not predict responsiveness of micrometastases.[402] Data from leukemia trials indicate that drugs active in the adjuvant (maintenance) setting may not be those that are most active against bulky macroscopic disease. Similarly, experimental data indicate that drugs with only modest activity against macroscopic tumor still may be active in the adjuvant setting. Finally, prolonged exposure to presurgical chemotherapy might select for drug-resistant tumor cells that might metastasize before definitive surgery.

The strategy of individualizing therapy on the basis of tumor response was pioneered at the Memorial Hospital in the T-10 trial.[185,384] Patients whose tumors demonstrated less than 10% residual viable tumor cells were continued on the same therapy as used preoperatively; patients with more than 10% residual viable tumor cells in the primary tumor were treated with a change in the postoperative chemotherapy. In preliminary reports of results of this study, almost all the patients demonstrating *favorable* chemotherapy effect were projected to survive free of recurrence. Nearly 85% of patients who demonstrated an *unfavorable* chemotherapy effect were projected to remain relapse-free at 3 years with the change in postoperative chemotherapy. The preliminary projected 3-year relapse-free survival rate for all patients in the T-10 study was 90%, with individualized therapy based on histologic assessment of residual viable tumor cells after presurgical chemotherapy. Because of these very favorable preliminary results, the T-10 protocol served as a model for many of the osteosarcoma treatment studies launched in the 1980s, virtually all of which featured the use of presurgical chemotherapy and individualization of therapy based on chemotherapy effect in the primary tumor.

Results reported from representative trials using presurgical chemotherapy are summarized in Table 35-4. Responses in the primary tumor have been variable, with favorable chemotherapy effect in 30% to 85% of patients. The overall results are excellent but comparable to adjuvant studies that used regimens of equal intensity without any presurgical chemotherapy (Table 35-2). Further, the importance of “custom tailoring” of therapy (whereby postoperative chemotherapy is individualized on the basis of assessment of chemotherapy effect in the primary tumor) in this strategy remains to be defined. Several trials are pertinent in this regard. The CCG attempted to duplicate the T-10 regimen in a multi-institutional setting (CCG 782).[394] Whereas patients achieving favorable chemotherapy effect in the primary tumor (28% of the patients) fared extremely well (projected 5-year event-free survival of 87%), the remaining patients with unfavorable chemotherapy effect did not benefit from a change in therapy postoperatively and had a less favorable outcome (projected 5-year event-free survival of 49%). Overall, only 56% of patients in the CCG study were projected to remain free of recurrent disease at 5 years—a disappointing result when compared to the initial results reported from Memorial Hospital.

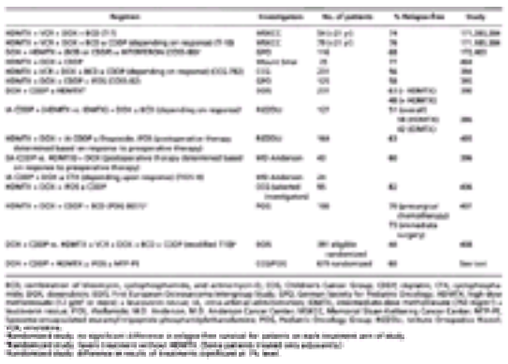


**TABLE 35-4. REPORTED RESULTS OF REPRESENTATIVE TRIALS INCORPORATING PRESURGICAL CHEMOTHERAPY FOR OSTEOSARCOMA**

Other studies conducted in the 1980s attempted to reproduce the remarkable results of the T-10 regimen, but all reported less promising results, particularly among patients achieving an unfavorable chemotherapy effect after presurgical chemotherapy. [389,408] In the COSS-82trial of the GPO,[395] which also tested the strategy of individualized therapy, results suggested that patients demonstrating unfavorable chemotherapy effect in the primary tumor are destined to do poorly and that treatment of poor responders with “salvage regimens” (as in the T-10 protocol) is inadequate to improve their prognosis. Investigators of the GPO concluded that active agents should not be withheld from the initial therapy of patients with newly diagnosed disease. At the Instituto Rizzoli, [396,409] overall results have improved over time, concurrent with the adoption of the strategy of presurgical chemotherapy. However, the Rizzoli investigators concluded that the improvement in prognosis more likely reflects improved effectiveness of the agents used rather than the use of presurgical chemotherapy per se, because a group of patients treated concurrently at the same institution without the benefits of presurgical chemotherapy fared just as well as did patients treated with presurgical chemotherapy. [409]

As in most trials of presurgical chemotherapy, an early trial of presurgical chemotherapy reported from the Rizzoli Institute demonstrated that patients achieving favorable chemotherapy effect in their primary tumors had a better overall outcome; however, change in the postoperative chemotherapy for those with unfavorable chemotherapy effect did not alter their adverse prognosis. However, in a more recent Rizzoli trial [405] conducted between 1986 and 1990, patients were treated initially with HDMTX, doxorubicin, and intra-arterial cisplatin preoperatively. Patients with favorable chemotherapy effect (less than 10% residual viable cells in their primary tumors) were treated postoperatively with 21 additional weeks of the same agents, whereas those with unfavorable chemotherapy effect received 30 weeks of chemotherapy, including ifosfamide and etoposide in addition to doxorubicin, cisplatin, and HDMTX. Seventy-one percent of patients in this study achieved a favorable chemotherapy effect after presurgical therapy, and 71% of those patients were projected to be disease-free survivors at 5 years. Of note, the patients with unfavorable chemotherapy effect had a projected disease-free survival equal to that of good responders if only patients receiving adequate therapy are considered. This Rizzoli trial represents one of the few studies in which the strategy of salvage chemotherapy for poorly responding patients has been shown to be of benefit.

Perhaps of greatest significance is that mature results from the Memorial Hospital studies [171,184] indicate that the very promising preliminary results were not sustained with further follow-up. Moreover, no difference in overall disease-free survival is apparent, regardless of whether patients received presurgical chemotherapy as part of their management. Although histologic evaluation of the primary tumor after presurgical chemotherapy strongly predicted subsequent disease-free survival and overall survival, with longer follow-up the Memorial investigators were unable to demonstrate an improvement in disease-free survival for patients who had unfavorable chemotherapy effect and received a modification of their postoperative chemotherapy as compared to a similar group of patients treated without such individualization of therapy. [171] In support of these findings, members of the Pediatric Oncology Group (POG) reported results of a randomized trial designed to test the impact of presurgical chemotherapy on outcome of patients with extremity osteosarcoma.[407] The overall event-free survival was identical regardless of whether patients were treated with chemotherapy prior to definitive surgery of the primary tumor. Moreover, the overall results of this study were identical to those achieved in a predecessor study wherein all patients were treated with immediate surgery followed by conventional adjuvant chemotherapy. Thus, the administration of presurgical chemotherapy (with or without individualized therapy on the basis of tumor response) per se does not appear to have led to an improvement in the outcome of children with osteosarcoma, at least in terms of rate of cure. Rather, recent improvements in outcome probably reflect the increasing intensity of the chemotherapeutic regimens used.

Although histologic evaluation of chemotherapy effect in the primary tumor after presurgical therapy is a powerful predictor of outcome, the likelihood that an individual patient will respond favorably cannot be predicted at the time of diagnosis. Because it is apparent that the majority of patients achieving a poor chemotherapy effect will relapse and modifications of postsurgical chemotherapy fail to have an impact on this unfavorable outcome, strategies are needed to predict favorably and poorly responding patients prior to the initiation of therapy so that more aggressive approaches can be used earlier in therapy for patients with a poor prognosis. The availability of reliable prognostic factors would permit stratification of patients to achieve two important goals: (a) selection of patients with an inferior prognosis who might benefit from novel or more intensive therapy, and (b) selection of patients with a superior prognosis who could be spared the acute and delayed toxicity of intensified therapy. Molecular markers that might accurately predict the response to presurgical chemotherapy and overall outcome currently are under study but cannot yet be recommended.[151,153,180]

The use of presurgical chemotherapy has been advocated also to increase the proportion of patients who are suitable candidates for limb salvage surgery and to facilitate limb-sparing procedures by tumor shrinkage in response to chemotherapy [410] and by provision of a window of time to fabricate customized endoprosthetic devices if needed for limb reconstruction. As noted, the largest proportion of limb-sparing procedures is performed at institutions using presurgical chemotherapy, but this finding may reflect the preference of institutional surgeons committed to limb-sparing surgery in any case, rather than reflecting any measurable benefit attributable to the use of presurgical chemotherapy per se. Little question remains that the number of patients believed to be suitable candidates for limb-sparing surgery is increasing concomitant with the increasing use of presurgical chemotherapy. This finding may reflect improvements in reconstructive techniques available and the increased experience and confidence of tumor surgeons. This trend has raised concern that selection criteria may be stretched by inexperienced surgeons to accommodate inappropriate candidates who might be better treated by amputation.[411] Presurgical chemotherapy (or for that matter, postoperative adjuvant chemotherapy) cannot reliably serve as a substitute for use of sound surgical principles.

Presurgical chemotherapy has been administered also via the intra-arterial route directly into the arterial supply of the tumor to maximize drug delivery to the tumor vasculature and drug extraction by the tumor.[386,397,412,413] Doxorubicin and cisplatin, in particular, have been delivered by prolonged intra-arterial infusion into vessels of the extremity. High local drug concentrations have been documented by pharmacokinetic studies, [397] and dramatic responses in primary tumors have been observed. The technique appropriately has been limited to centers with excellent angiographic support and facilities to administer repeated courses of intra-arterial therapy preoperatively. The rationale for the use of intra-arterial therapy is not self-evident for several reasons. Even in the prechemotherapy era, control of the primary tumor was rarely a problem. Rather, micrometastatic disease present in the lung ultimately killed the patient. Improvements in the outcome of patients with osteosarcoma have resulted directly from improvements in systemic chemotherapy for micrometastatic disease rather than from better local control measures. Thus, strategies that improve drug delivery to the primary tumor at the expense of drug delivery to micrometastatic disease are counterintuitive. Further, most intra-arterial regimens use single-agent chemotherapy for the first 2 to 3 months of treatment, whereas improvements in outcome for patients with osteosarcoma have resulted from the application of multi-agent chemotherapy.

Data from the COSS-86 study of the GPO challenge the notion that intra-arterial administration leads to superior response in the primary tumor [414] as compared to the same treatment given intravenously. Also unclear is whether the administration of intra-arterial chemotherapy translates into superior overall outcome for patients as determined by improvement in relapse-free survival. An update of studies from the M. D. Anderson Cancer Center, where intra-arterial chemotherapy was pioneered,[396] indicates that the overall disease-free survival for patients treated with intra-arterial cisplatin, definitive surgery, and postoperative adjuvant chemotherapy is projected to be 60%, a disappointing result from a single-institution trial when compared to results achieved in other multi-institutional trials with or without presurgical chemotherapy (see Table 35-2, Table 35-4). Even if the administration of intra-arterial chemotherapy does not improve the overall outcome for patients with osteosarcoma, intra-arterial administration of presurgical chemotherapy has been suggested possibly to maximize response in the primary tumor, thus facilitating limb-sparing surgical procedures, and to maximize the number of patients suitable for such procedures. A study conducted at the Rizzoli Institute [415] addressed this issue directly by randomly assigning patients with osteosarcoma to receive presurgical chemotherapy (HDMTX, doxorubicin, and cisplatin) intravenously or to receive the same presurgical chemotherapy with intra-arterial administration of cisplatin. Although the proportion of patients achieving favorable responses in the resected tumor specimens was significantly higher among patients treated with intra-arterial therapy, the proportion of patients treated with limb-sparing surgery was the same in the two treatment groups (intravenous and intra-arterial therapy). Thus, from the perspectives of overall cure rate and rate of

limb-sparing surgery, the administration of intra-arterial chemotherapy does not seem justified, and the popularity of this strategy has waned in recent years.

### *Use of Biologic Response Modifiers*

Therapeutic trials based on immunologic approaches to osteosarcoma have been stimulated by documentation of tumor-specific humoral [17,19] and cellular [20] immune responses in patients and animals with osteosarcoma. The presence in osteosarcoma patients of tumor-specific cytotoxic lymphocytes that are inhibited by a concomitant population of "inhibitor lymphocytes" [20] suggestsa role for specific and nonspecific immune stimulation in the treatment of osteosarcoma. Early trials using the injection of inactivated osteosarcoma cells [416] or tumor cell lysates [417] induced evidence of cellular immune response in patients but produced no definitive therapeutic advantage. Similarly, cross-transplantation of biopsy specimens as a means of antitumor immunization resulted in no significant reduction in the incidence of metastatic disease. [416,418] Nonspecific immune stimulation with bacille Calmette-Guérin vaccine was not effective therapeutically, [416] even when administered in conjunction with adjuvant chemotherapy. [370] Interferon has been demonstrated to inhibit the growth of osteosarcoma cell lines *in vitro* [419] and was used as an adjuvant to surgery in an uncontrolled trial from the Karolinska Hospital in Sweden. A significant improvement over historical results was observed, but this improvement was less dramatic when compared to a concurrent group of patients treated only with surgery in other Swedish hospitals. [114,420] In the COSS-80 trial (Table 35-4), patients were treated with presurgical and postsurgical adjuvant chemotherapy and were assigned randomly to receive or not to receive interferon in addition to chemotherapy. No benefit in relapse-free survival could be demonstrated for patients treated with interferon. [172]

Liposome-encapsulated muramyl tripeptide-phosphatidylethanolamine (liposomal MTP-PE), a derivative of bacille Calmette-Guérin, is a promising biologic agent for the treatment of osteosarcoma. [421,422] and [423] Liposomal MTP-PE activates monocytes *in vitro* to a tumoricidal state against osteosarcoma and other tumors. When it is injected intravenously in animals, similar macrophage activation can be demonstrated, resulting in the complete eradication of established pulmonary metastases in a rodent model. In dogs with spontaneously occurring osteosarcoma, the administration of MTP-PE prolonged median survival after amputation as compared to untreated controls, [424] and the combination of cisplatin and liposomal MTP-PE resulted in superior survival of dogs with osteosarcoma as compared to those treated with cisplatin alone. [425,426] Studies to date indicate that liposomal MTP-PE can be administered safely to human beings, [422,423] but the efficacy of the compound in the treatment of osteosarcoma awaits further study. Likely, liposomal MTP-PE will be most useful in patients with minimal residual disease, probably in combination with standard chemotherapy. A randomized trial testing this hypothesis recently was conducted in the United States and Canada jointly by the CCG and the POG, with promising preliminary results. [457]

Hybridoma technology and advances in the technology for cloning T cells and expanding such clones for therapeutic purposes [427] provide interesting possibilities for future trials. Monoclonal antibodies specific for the ganglioside $G_{D2}$—a cell-surface antigen expressed by human neuroblastomas—also recognize human osteosarcomas. [428] These antibodies already have been used for imaging and treatment of patients with neuroblastoma and may prove useful in treatment of patients with osteosarcoma as well. Clinical trials of such antibodies now are under way. Similarly, T-cell clones that have been isolated from the peripheral blood of patients with osteosarcoma [429] are cytotoxic to autologous tumors and can be expanded *in vitro* for reinfusion into patients. Possibly, lymphocytes with enhanced antitumor reactivity may be cloned directly from tumor specimens [427] and may provide a more potent source of immunoreactive cells for therapy of osteosarcoma.

### *Treatment of Metastases*

Historically, patients who had osteosarcoma and developed metastases had a poor prognosis and were treated palliatively. Most of such patients died within 1 year of developing metastatic disease. Although attempts at surgical resection of metastatic pulmonary nodules were undertaken as early as 1940, [430] the first systematic aggressive surgical approaches to these patients were initiated in the mid-1960s. Little doubt remains that the development in the last 20 years of more effective salvage therapies for patients who develop metastases has contributed substantially to the improved survival of children with osteosarcoma. [430,431,432,433,434,435,436,437,438,439,440,441,442,443,444] and [445]

The biology of osteosarcoma offers the unique opportunity to cure patients who have developed metastases. More than 85% of recurrences are in the lung, where complete surgical resection of tumor nodules with wide margins can be accomplished relatively easily (and repeatedly). With frequent thoracic CT scanning, metastatic nodules can be detected when fairly small and more easily resectable, although in most cases the surgeon will discover at thoracotomy more lesions than anticipated from the CT scan. [446] Not all lesions detected on CT scan represent metastases, [447] and histologic confirmation should be undertaken (at least for a first relapse) before committing affected patients to intensive salvage therapy. In a significant proportion of patients, the lungs are likely to be the *only* site of metastases, especially when recurrences appear relatively late (i.e., more than 1 year after diagnosis) and when the metastatic lesion is solitary. In such cases, the recurrent tumors are likely to have a more indolent behavior and may not themselves further metastasize. Such patients have been cured by thoracotomy alone (discussed later). [448]

Complete surgical resection of all overt metastatic disease is a prerequisite for long-term salvage after relapse. Patients not treated by thoracotomy have little hope for cure. [436,437,443,444] The majority of pulmonary lesions can be resected successfully by wedge resection without risk of local recurrence [432] while preserving the maximum lung tissue and allowing for the possibility of future pulmonary resections. Rarely, segmentectomy, lobectomy, or even pneumonectomy may be required to control more extensive lesions. Bilateral lung metastases can be approached by staged lateral thoracotomies performed 1 to 2 weeks apart, a program that is tolerated well by otherwise healthy young adults and adolescents.

Some surgeons have advocated an approach by median sternotomy. Although metastases can be removed from both lungs in one procedure using this approach, surgical exposure is not as complete (especially in the retrocardiac area and left lower lobe), and subsequent repeat thoracotomy is more difficult.

The completeness of surgical resection is an important determinant of outcome in that patients left with measurable or microscopic disease at the resection margins are unlikely to be cured. [436,449] Evidence of disruption of the visceral pleura by tumor has been found to be associated with an adverse prognosis and is thought to carry the same implications as incomplete resection. [437,441] In one series, 9 of 11 (82%) patients who had complete resections without evidence of pleural disruption became long-term survivors, as compared to only 2 of 15 (13%) who had incomplete resection or pleural disruption by tumor. More stringent criteria for complete resection, which include attention to the status of the pleura, are valuable prognostically but, even with an aggressive surgical approach, only 42% of patients (11 of 26) could be rendered disease-free surgically in one series when these stringent criteria were applied. [437]

Several other prognostic factors have been examined in patients treated for metastases. Variables that appear to have significant prognostic value include the number of nodules detected on the prethoracotomy CT scan and the disease-free interval between initial diagnosis and the development of metastases. In general, patients with late-appearing solitary pulmonary nodules (more than 1 year after surgery for the primary tumor) are most likely to be cured, whereas those with more than three nodules that appear within 6 months of surgery have a less favorable outcome. [432,436,437,443,445,448,449] Although an initial disease-free interval of less than 6 months indicates a less favorable prognosis, this finding should not exclude affected patients from consideration for salvage surgery [436]; the poor prognosis for patients who experience relapse within 1 year of initial diagnosis reported in some series may reflect the use of a less aggressive approach for such patients. [437] The number of nodules also has not been prognostically important in all series, although patients presenting with more than 16 nodules on preoperative tomography are unlikely to have successful complete resection. [436] Several reports indicate that adjuvant chemotherapy for primary osteosarcoma may reduce the number of metastatic nodules in patients destined to relapse, thus increasing the chance that these metastases will be resectable. [110,450] However, that this translates into an improved cure rate for patients developing recurrent disease after adjuvant chemotherapy is unclear. [450]

Many investigators have advocated the use of postthoracotomy adjuvant chemotherapy (and even lung irradiation) [438,440,443,444] and [445] to destroy presumed residual microscopic deposits after surgical treatment of overt metastases. The contribution of such adjuvant therapy has not been examined in a controlled study. In small series, long-term survival has been reported without the use of further chemotherapy, [436,437,441,448] although survivors treated with only surgery were more likely to be those who had suffered late relapse with solitary pulmonary nodules. The likelihood that a patient will benefit from the administration of chemotherapy to treat relapse is highly dependent on the chemotherapy experience of such a patient prior to relapse. Tumors recurring after exposure to multi-agent intensive regimens are less likely to respond to salvage regimens (assuming that active drugs can even be identified that have not already been used in the previous treatment of the patient).

Thus, when overt metastatic disease is discovered, a systematic approach is recommended. A careful search for all metastatic lesions by thoracic CT scan and radionuclide bone scan is essential; other investigations to search for metastases to other sites should be performed if clinically indicated. The discovery of unresectable extrathoracic metastases or of obviously unresectable pulmonary disease (because of hilar involvement, malignant pleural effusion, massive disease or, perhaps, the presence of more than 16 nodules) is a contraindication to aggressive thoracotomy, and the patient should be treated palliatively. Radiotherapy may be

particularly useful in palliative treatment. In selected patients with unresectable disease (especially those with no previous exposure to chemotherapy), an aggressive approach with curative intent still may be indicated. The use of chemotherapy (with or without radiotherapy) rarely produces complete response of all metastatic disease, but some patients with inoperable metastases may respond sufficiently to permit complete resection of disease at a later date, with a chance for long-term disease control. Rare patients with isolated bony metastases also have been cured by such an aggressive approach.

Patients with resectable lung disease should undergo thoracotomy to remove all evidence of disease. Bilateral disease is not a contraindication and can be approached by staged bilateral thoracotomies or median sternotomy. The role of adjuvant chemotherapy after thoracotomy remains to be defined, but such treatment probably is indicated, at least for patients with multiple lesions (more than three) appearing within 6 months to 1 year of initial surgery and for patients who have incomplete resection of metastatic disease or evidence of pleural disruption by tumor. Repeat thoracotomies may be required for subsequent recurrence and should be recommended as long as all evidence of disease can be resected.

Several approaches incorporating chemotherapy and radiotherapy administered before or after thoracotomy have been used to treat patients with metastases. Survival after relapse doubtless has been enhanced by approaches designed with curative intent that incorporate repeated aggressive surgery to remove overt disease. With such treatment, 30% to 40% of patients have been reported to survive beyond 5 years after relapse, [435,436] and [437,440,441,443,444] and [445] although not all these patients ultimately will be cured. Many of these reports include patients who did not receive adjuvant chemotherapy as part of their initial treatment or whose adjuvant chemotherapy did not include all agents with demonstrable activity. Patients who relapse after multi-agent chemotherapy and surgery have a significantly lower probability for survival. Such considerations emphasize the value of close follow-up, with frequent chest radiographs and thoracic CT scans to detect recurrent disease when it still is resectable. Thus, for patients with osteosarcoma, presentation with or development of metastases is not a hopeless situation; aggressive systematic treatment of metastases offers prolonged survival for many patients and the possibility of cure for a significant fraction. Ironically, as adjuvant regimens used in frontline therapy of patients are intensified and the number of patients surviving without ever developing recurrence increases, the proportion who are likely to be salvageable after relapse may decrease, because patients who experience relapse are more likely to have drug-resistant recurrences.

Management of patients with clinically detectable metastatic disease at initial presentation incorporates some of the same principles that guide the management of recurrent disease.[451,452] and [453] Such patients have no previous exposure to chemotherapy, and their tumors thus are more likely to be chemosensitive than are those tumors recurring after initial adjuvant therapy. As in the treatment of recurrent disease, resection of all measurable disease (at the primary and metastatic sites) is a prerequisite for cure, and patients with extrathoracic metastases are unlikely to be cured. Timing of the surgery of the primary tumor and metastatic sites has been variable, but most modern approaches prescribe a strategy of alternating chemotherapy and surgery. The initial treatment usually is a course of chemotherapy, followed by surgical resection of the primary tumor, followed by a second course of chemotherapy and surgical ablation of metastatic sites, followed by the remaining courses of chemotherapy. Patients with tumors that are responsive to presurgical chemotherapy are more likely to be cured. Although improving, the outlook for patients presenting with metastatic disease is poor.

## CONCLUSIONS AND FUTURE CONSIDERATIONS

In the last three decades, the prognosis for children with osteosarcoma has improved dramatically. The benefit of adjuvant chemotherapy that prevents recurrence in almost two-thirds of patients with limb primaries is indisputable. Aggressive, systematic approaches with thoracotomy have improved the outlook for patients presenting with or developing metastases after therapy. Nevertheless, refinements in therapy are needed. More than one-third of children presenting without metastases will experience relapse after current therapy. The strategy of presurgical chemotherapy has not lived up to the initial promise of improving the disease-free survival for patients with osteosarcoma, although this strategy may facilitate limb-sparing surgery.

The morbidity related to therapy is considerable because many patients still require amputation to control the primary tumor; advances in surgical techniques for limb reconstruction may allow for limb-sparing surgery for an increasing proportion of patients. The toxicity and expense of current chemotherapeutic regimens are substantial, and the late effects of such therapy have not yet been assessed completely. The late cardiac injury resulting from therapy with anthracyclines already has emerged as a significant price of cure for survivors of osteosarcoma. [454] Despite promising new approaches, patients with axial skeleton primaries continue to fare poorly because local control cannot be achieved in the majority of cases. The outcome for patients presenting with metastatic disease remains unsatisfactory.

Limb salvage has been adopted enthusiastically by orthopedic surgeons. Although the safety of this approach appears to have been demonstrated and the functional results appear to be superior to amputation, late complications occur in many patients, and the psychological outcome for patients undergoing limb salvage is not clearly superior to that of amputees.[193,213] Limb-sparing surgery thus may not be the appropriate approach for all patients. Strategies to sterilize the primary tumor site by administering intensive intra-arterial chemotherapy in the hope of avoiding surgery for the primary tumor altogether have been attempted, but results to date indicate an unacceptable local recurrence rate, [396] and this approach cannot be recommended.

New active drugs promise to add further to the armamentarium of chemotherapeutic agents available for use against osteosarcoma. Ifosfamide already has been incorporated into frontline treatment regimens, although the benefit of this addition remains to be confirmed. Primary drug resistance and treatment failure in osteosarcoma may be associated with the multi-drug-resistant phenotype mediated by p-glycoprotein. Strategies to reverse the multi-drug-resistant phenotype with cyclosporine and its less toxic analogs may prove useful in the management of recurrent disease and, ultimately, in frontline treatment.

Although immunotherapy of osteosarcoma has not yet proved successful, advances in technology have provided immunotherapists with more active and more specific reagents. Monoclonal antibodies against osteosarcoma may prove useful for delivering drugs or radiopharmaceuticals directly to tumor. Antibodies with exquisite specificity will be required for this purpose. Cloned cytotoxic T cells may also provide more specific antitumor therapy and may prove most useful in the adjuvant setting.

Finally, studies of osteosarcoma have elucidated some of the growth factors and receptors that play critical roles in tumor cell proliferation. Insulin-like growth factor-1 has been found to be a potent mitogen for osteosarcoma cells, suggesting possible intriguing endocrine therapies of this disease. [455] Osteocalcin is expressed in high levels by tumor cells of osteosarcoma, suggesting the use of the osteocalcin promoter as a relatively tissue-specific target for delivery of therapeutic toxic genes (e.g., the herpes simplex thymidine kinase gene that activates acyclovir to produce cytotoxicity in dividing cells). [456] Overexpression of HER2 in a subset of patients with osteosarcoma with adverse prognosis[181,182] suggests a strategy to target this receptor therapeutically. A “humanized” antibody directed against the extracellular domain of the HER2 receptor (Herceptin) already has proven to be effective in women with advanced breast cancer and currently is under study in a trial for patients with metastatic osteosarcoma.

The prospects for understanding the biology of osteosarcoma and improving therapy for affected children thus appear bright. Although numerous problems remain to be addressed, the current state of therapy—with more than two-thirds of patients who have nonmetastatic osteosarcoma of the extremity being cured of their disease—represents an exceptional advance, achieved in less than three decades. The celebrated surgeon of the 1920s who exhorted his colleagues to pray for children with osteosarcoma might well be gratified by the remarkable advances in treatment over the last 30 years.

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# GERM CELL TUMORS

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## INTRODUCTION

Gonadal and extragonadal germ cell tumors are infrequent in childhood, occurring at a rate of 2.4 cases per million children and representing approximately 1% of cancers diagnosed in persons younger than 15 years.[1] Until recently, the myriad of histologic subtypes and sites of origin and the paucity of cases hindered the standardization of care for such children. The challenge presented by these neoplasms to pediatric oncologists and pediatric surgeons is to control the tumor while maintaining future fertility. With the advent of effective chemotherapy and the recognition that many of these tumors respond in a similar manner, the management of germ cell tumors is being clarified. However, because of the low incidence of these tumors, cooperative group studies will be necessary to extend future therapeutic advances.

## EMBRYOGENESIS AND HISTOGENESIS OF GONADAL TUMORS

Germ cell tumors are presumed to share a common cell of origin, the primordial germ cell, yet they remain a heterogeneous group of tumors. Variations regarding age, sites of presentation, histopathology, and malignant potential stem from differences in the stage of germ cell development at tumorigenesis, differences in the tumor environment secondary to the gender of the patient and location of the clone, and specific genetic aberrations. Therefore, understanding the development of embryonic germ cells is critical to an appreciation of these issues.

The primordial germ cells first become evident in the extraembryonic yolk sac by the fourth week of gestation. By the fifth week, the germ cells migrate through the mesentery to the gonadal ridge.[2] This migration appears to be mediated by the c-kit receptor and its ligand, stem-cell factor, or steel factor. Primordial germ cells express c-kit. Stem-cell factor is expressed with an increasing gradient from yolk sac to gonadal ridge, guiding germ cells to the gonadal ridge. [3,4] and [5] In animal models, primordial germ cells not expressing c-kit are unable either to migrate to the gonad or to proliferate during this migration. Extragonadal germ cell tumors are presumed to arise from germ cells that have migrated aberrantly.[6]

The fate of the germ cells, after their arrival at the gonadal ridge, depends on the sex genotype of the individual. During a narrow window of opportunity in the sixth to seventh week, a gene on the Y chromosome (the SRY gene) initiates male sex determination. Ovarian differentiation commences either in the absence of a Y chromosome or if the window of opportunity is missed.[7] Testicular differentiation manifests itself by the abrupt development of cellular cords (sex cords) in the seventh week. Primordial germ cells populate these sex cords; then, within a few days, they undergo mitotic arrest and remain until puberty. The ovary does not demonstrate the defined sex cords seen in the testis. Instead, primordial germ cells populate the mesenchyme of the primitive gonad while continuing to divide and proliferate. At approximately 16 to 18 weeks' gestation, the germ cells gradually enter into meiosis I, then arrest in the prophase of meiosis I as oocytes. Simultaneously, the follicular cells surround the oocytes. Primordial germ cells are not associated with follicular cells prior to entry into meiosis. The entry into meiosis of primordial germ cells is a gradual process that continues until birth.

The gonads contain three cell types having neoplastic potential. Germ cells give rise to germ cell tumors. The cells of the sex cords rarely may develop into stromal tumors, such as testicular Sertoli or Leydig cell tumors, ovarian granulosa cell tumors, or mixtures of these components. Last, coelomic epithelium covering the ovary may evolve into epithelial neoplasms, found most often in adults.[8,9]

## GENETICS AND MOLECULAR BIOLOGY

The heterogeneity of the pediatric germ cell tumors is evident also in studies investigating their genetic and molecular properties. Four biologically distinct subcategories are noted: tumors of the adolescent testis, tumors of infancy, extragonadal tumors of adolescents, and tumors of the adolescent ovary.

### Genetic Characteristics of Adolescent Testicular Tumors

Adolescent testicular germ cell tumors most commonly become clinically evident several years after puberty, suggesting that a critical genetic event occurs with, or is unmasked at, puberty. However, because these tumors have been shown to arise in premeiotic germ cells, some observers believe the critical event occurs in the embryonic gonad.[10] Despite their histologic heterogeneity, tumors of the adolescent and adult testis are relatively homogeneous genetically, demonstrating an aneuploid DNA content and the isochromosome 12p or i(12p).[11,12,13 and 14]

The i(12p) is composed of two copies of 12p (Fig. 36-1), both from the same parental origin.[15] Testicular tumors lacking i(12p) often show gain of 12p material within marker chromosomes.[16] The i(12p) has been documented by fluorescent *in situ* hybridization in intratubular germ cell neoplasia, a precursor lesion of testicular germ cell tumors. This finding provides further evidence that this genetic alteration occurs early in germ cell tumor pathogenesis.[17] Whether the critical genetic event is gain of 12p, loss of 12q, or both is not fully understood. Murty et al.[18] noted loss of heterozygosity at the 12q13 and 12q22 regions in 41% and 47% of tumors, respectively. Genes implicated in the pathogenesis of other solid tumors that map to the 12q13 region, including INT1, GLI, and MDM2, are not altered in testicular germ cell tumors.[18] Testicular germ cell tumors also have exhibited loss of chromosome 13 (38%), gain of chromosome 21 (45%), gain of chromosome 8 (45%), gain of chromosome 1q (36%), and high-level gain of 12p11.2-12.1, suggesting amplification.[19,20] Other less frequent genetic changes include loss of 1p,[21,22] K-ras, and N-ras mutations[23]; high N-*myc* expression without amplification[24]; absence of p53 mutation[25]; loss of heterozygosity for 11p13 and 11p15 with preferential loss of the paternal allele[26]; loss of 3p[27]; deletion of DCC[28,29]; and biallelic expression of H19 and insulin-like growth factor-2.[30]

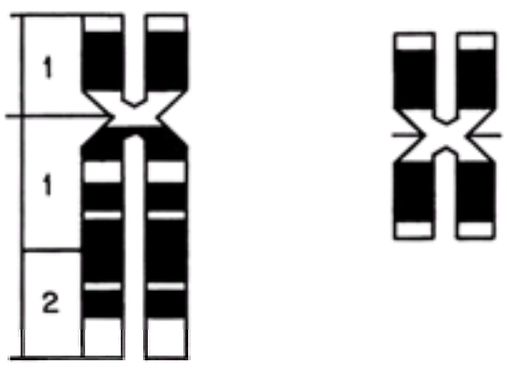


**FIGURE 36-1.** Normal chromosome 12 (*left*) and isochromosome 12p (*right*).

### Genetic Characteristics of Adolescent Ovarian Tumors

The genetic biology of ovarian germ cell tumors is more complex than that of testicular germ cell tumors and is considered separately for mature teratomas, immature teratomas, and malignant ovarian germ cell tumors.

#### *Mature Teratomas*

Cytogenetic assessment of more than 325 ovarian mature teratomas demonstrates that 95% are karyotypically normal, with only 5% showing gains of single whole chromosomes, the identity of which differs from case to case.[31,32,33 and 34] Studies of molecular loci have shown that the majority of mature ovarian teratomas have entered but not completed meiosis.[31,35,36] These diploid tumors are genetically unique in that the majority of their genome is isodisomic.[37]

#### *Immature Teratomas*

Ovarian immature teratomas are heterogeneous. Some show evidence of a meiotic stem-cell origin, and others show mitotic origins, suggesting failure of early meiotic arrest.[38] The frequency of chromosomal abnormalities in immature teratoma is higher than in mature teratoma, but no consistent abnormalities, including the i(12p), have been described. All except one of the patients with cytogenetically abnormal immature teratomas reported to date have experienced multiple recurrences. The exception was a tumor showing a 47,XXX karyotype. In contrast, all patients previously described with karyotypically normal immature teratomas have remained disease free.[38,39,40,41,42,43 and 44]

Apparently a correlation exists between the histologic grade of immature teratoma and DNA content. Grades 1 and 2 are diploid, and grade 3 tumors are aneuploid.[45] King et al.[39] reported that diploid, grade 3 immature teratomas may have a better prognosis as compared to aneuploid tumors.

#### *Malignant Ovarian Germ Cell Tumors*

As are germ cell tumors of the adolescent testis, most malignant ovarian germ cell tumors are aneuploid. Approximately 75% contain i(12p), 42% and 32% have gains of chromosomes 21 and 1q, respectively, and 25% and 42% have loss of chromosomes 13 and 8, respectively.[44,46,47,48,49,50 and 51] DNA fingerprinting of four patients with dysgerminoma suggested an origin from premeiotic germ cells or germ cells at the beginning of the first meiotic division.[35] Also described was the development of a malignant endodermal sinus tumor with i(12p) and aneuploidy within an immature teratoma that had no i(12p) and was diploid. Such an event supports a genetic clonal evolution in this process.[46,47]

In summary, although malignant ovarian germ cell tumors appear to be equivalent to their adolescent testicular counterparts, immature and mature ovarian teratoma remain unique subcategories of germ cell tumors likely to have a different mechanism of origin.

### Genetic Characteristics of Extragonadal Germ Cell Tumors of Older Children

Aberrant or incomplete migration of primordial germ cells is one explanation for the origin of extragonadal germ cell tumors. Another hypothesis is that these tumors arise from totipotent embryonal cells that have escaped the influence of embryonic organizers controlling normal differentiation. This latter proposal is supported by a number of observations, the foremost being that ectopic germ cells only rarely have been reported to exist in human embryos, most having disappeared by 18 weeks' gestation.[52,53 and 54] Although data from animal models predict these tumors to be postmeiotic in origin,[55] other studies have shown extragonadal germ cell tumors in older children to be postmitotic in origin.[48,56,57 and 58]

The two most common sites for extragonadal germ cell tumors in older children are mediastinum and brain. Cytogenetic analysis of central nervous system teratoma has shown a high frequency of sex chromosome abnormalities, most commonly increased copies of the X chromosome.[59,60] The i(12p) has been described in some, but not all, pineal germinomas, but it has not been seen in pineal teratoma.[60,61 and 62] Ploidy analyses of mediastinal germ cell tumors suggest that most are diploid or tetraploid,[63] and some contain i(12p).[64] The extragonadal germ cell tumors in adolescents and adults are associated with hematopoietic malignancies of various cell lineages that present soon after the initial presentation of the germ cell tumors. The malignant hematopoietic clone commonly demonstrates i(12p), unlike hematopoietic malignancies that arise secondary to therapy.[65,66 and 67]

### Genetic Characteristics of Extragonadal and Testicular Germ Cell Tumors of Young Children

In children younger than 4 years, germ cell tumors arising in gonadal and extragonadal sites are histologically, clinically, and genetically similar. Most teratomas in this age group are diploid, have normal karyotypes and, if completely resected, behave in a benign fashion regardless of degree of immaturity and site of origin.[48,56,57,68,69] Malignant germ cell tumors in these young children are almost exclusively yolk sac tumors, arise from a preexisting teratoma, and most often are diploid or tetraploid.[45,70] Recurrent cytogenetic abnormalities involve chromosomes 1, 3, and 6, among others, but only rarely the 12p.[45,69,70 and 71] *In situ* hybridization studies have demonstrated deletion of 1p36 in 80% to 100% of infantile malignant germ cell tumors arising from testicular and extragonadal sites.[72,73] Genetic surveys of regions of gain or loss in these infantile yolk sac tumors document recurrent loss of 6q24-qter, gain of 20q and 1q, and loss of 1p. A small minority of tumors show evidence for c-myc or n-myc amplification.[74] The clinical significance for these markers is entirely unknown. Patterns of genetic expression have not been reported in these tumors.

## PATHOLOGY

Germ cell tumors show numerous histologic subtypes. Although the histologic features of each subtype are independent of presenting clinical characteristics, tumor biology and clinical behavior vary with site of origin, stage, and age of the patient.[75,76] For example, mature teratoma in infants and in the ovary almost invariably are diploid and benign, whereas those in the adult testis are aneuploid and potentially malignant.[77] The histologic classification of these tumors is shown in Table 36-1. The pathologic features of each histologic subtype are discussed separately.

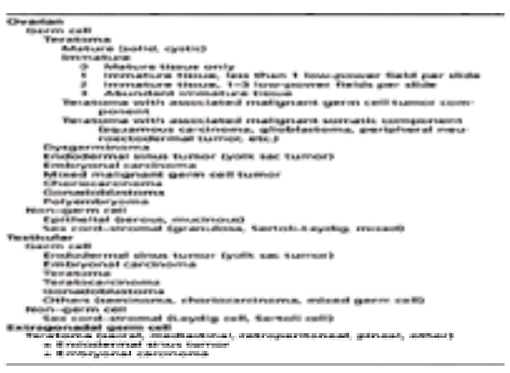


**TABLE 36-1. HISTOLOGIC CLASSIFICATION OF PEDIATRIC GONADAL AND EXTRAGONADAL TUMORS**

### Mature Teratoma

Teratomas are the most common histologic subtype of childhood germ cell tumor arising in ovary and extragonadal locations.[75,78,79 and 80] Mature teratomas of the gonads are encapsulated, multicystic, or solid tumors.[75,78,79 and 80] Extragonadal teratomas differ from those arising from the gonads only in the absence of a clearly defined external capsule. This characteristic in sacrococcygeal teratoma requires that the coccyx be removed during surgery to reduce the risk of recurrence.

The mature teratoma is composed of mature representative tissues from all three germ cell layers: ectoderm, mesoderm, and endoderm. Although any tissue type may be seen, the most common are skin and skin appendages, adipose tissue, mature brain, intestinal epithelium, and cystic structures lined by squamous, cuboidal, or flattened epithelium. Some tissue types are site-specific. For example, hematopoietic, pancreatic, or pituitary tissue frequently is found in mediastinal tumors and rarely in teratoma at other sites.[81] Components of the mature teratoma occasionally may be biologically active, with secretion of enzymes or hormones, including insulin, growth hormone, prolactin, and vasopressin.[82,83 and 84]

### Immature Teratoma

Immature teratomas have a gross appearance similar to that of mature teratoma and likewise are composed of representative tissues from all three germ layers. Unique to these tumors is the presence of various immature tissues, usually neuroepithelium, although immature ectodermal, mesodermal, and endodermal elements also may be observed. A number of grading systems have been established for immature teratoma, all of which systems are variations of the system originally devised by Thurlbeck and Scully.[85] All quantify the degree of immaturity in the lesion (Table 36-1). Only in immature teratoma of the adult ovary has grading of immature elements had potential prognostic significance. Even so, most of these studies predated the full appreciation of the range of histologic appearance of endodermal sinus tumor. Immature teratomas in children behave in a malignant fashion only if foci of malignant germ cell elements (usually yolk sac tumor) and specific clinical characteristics (usually advanced stage) are present. Because clusters of yolk sac tumor may be very small or associated intimately with the immature neural tissue, or both, and because they frequently do not stain positively for a-fetoprotein (AFP), they are easily overlooked ( Fig. 36-2). Tumors containing such foci likely are responsible for the reports that immature teratoma may metastasize.

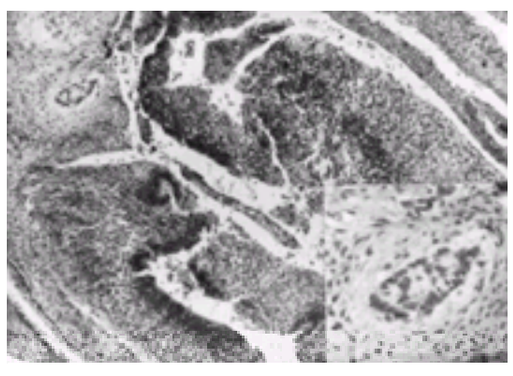

**FIGURE 36-2.** A small focus of glandular yolk sac tumor is present in a loose myxoid background (*upper left*), closely associated with a large area of immature neural tissue. In the inset, the large, vesicular nuclei and prominent nucleoli of the yolk sac tumor cells are seen at higher magnification.

### Yolk Sac Tumor (Endodermal Sinus Tumor)

Yolk sac tumors are the most common pure malignant germ cell tumor in young children and are the most common germ cell tumors, benign or malignant, in the testes of infants and young boys.[77] Yolk sac tumor is the only malignant germ cell tumor type occurring in the sacrococcygeal region.[75,80,86] Yolk sac tumors rarely occur in pure form in extragonadal sites of adolescents but more frequently are a component of the mixed malignant germ cell tumors occurring in these locations.[81,87,88] Grossly, these tumors consist of friable, pale gray, mucoid tissue in which variable amounts of hemorrhage and necrosis are present. The microscopic features are varied and have been characterized fully only in the last two decades.[89,90] Individual cells may be small and pale, with scant cytoplasm, round to oval nuclei, and inapparent nucleoli, or they may be medium-sized to large with clear vesicular nuclei and prominent nucleoli, resembling cells of an embryonal carcinoma or germinoma. Mitoses range from few to many. Four general patterns and a number of variations have been recognized. These patterns are useful in the recognition of endodermal sinus tumor but have no other known clinical relevance.

The pseudopapillary or festoon pattern and the microcystic or reticular pattern are the most common and widely recognized. Both contain Schiller-Duval bodies, structures formed by a central small blood vessel closely invested by two layers of tumor cells ( Fig. 36-3). The microcystic or reticular pattern is associated most often

with eosinophilic globules and strands that only occasionally stain positively for AFP or a $_1$-antitrypsin. The pseudopapillary and parietal patterns often are observed after chemotherapy.[91] The solid pattern usually is found only focally and may resemble embryonal carcinoma, but the cells are smaller and less pleomorphic than those of embryonal carcinoma and tend to have nucleoli less prominent than those of either embryonal carcinoma or germinoma. A variant of the solid pattern is the hepatoid pattern, which closely resembles fetal liver.[92] A fourth pattern is the polyvesicular vitelline pattern, characterized by small, empty cystic structures lined by a single layer of malignant cells that merge from cuboidal to flat. The cells often are embedded in a loose, frequently myxoid stroma. Two other patterns have been described. The enteric pattern resembles the fetal human gastrointestinal tract and typically stains positively for AFP and chorionic embryonic antigen.[90,93,94] The mesenchyme-like pattern stains positively for cytokeratin and vimentin but not for AFP and has been implicated as the source of the sarcomas that occasionally occur in patients who have had a yolk sac tumor.[92]

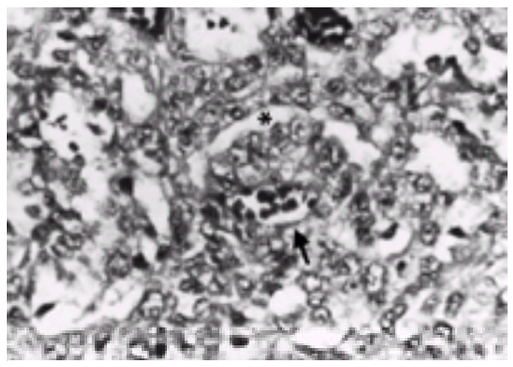

**FIGURE 36-3.** Schiller-Duval body seen here in the microcystic pattern of yolk sac tumor is characterized by a central blood vessel ( *arrow*) closely invested by a layer of tumor cells and separated from a second layer of tumor cells by a space (*star*), likened by some to Bowman's space in the glomerulus.

### Germinoma

Germinomas, also termed *dysgerminomas* or *seminomas*, depending on tumor location, are the most common pure malignant germ cell tumors that occur in the ovary (dysgerminomas) and central nervous system in children.[86,87,95] Pure seminomas represent the most common malignant germ cell tumor in men older than 20 years. However, pure seminomas are distinctly unusual in men younger than 20 years.[77] This distinction likely is due to the slower growth rate of seminomas and hence the later age of presentation. Grossly, germinomas are encapsulated, solid, gray-pink tumors with a rubbery consistency and occasional small foci of hemorrhage and necrosis. Microscopically, the tumor cells are arranged in nests separated by bands of fibrous tissue in which variable numbers of lymphocytes are identified ( Fig. 36-4). The cells are large, with clear cytoplasm, distinct cell membranes, and large round nuclei having one or two prominent nucleoli. Granulomas with giant cells frequently are present. Syncytiotrophoblasts also may be present, but they do not alter the prognosis of the tumor unless they are associated with cytotrophoblasts in foci of choriocarcinoma. Immunohistochemically, the germinoma cells have strong staining for placental alkaline phosphatase (PLAP), whereas the syncytiotrophoblasts stain for human chorionic gonadotropin beta-subunit (b-HCG). The primary differential diagnosis of germinomas, particularly in small biopsies of extragonadal sites and at the time of frozen section analysis, includes a hematopoietic disorder such as lymphoma and the solid variant of endodermal sinus tumor, as these merit different therapeutic approaches.

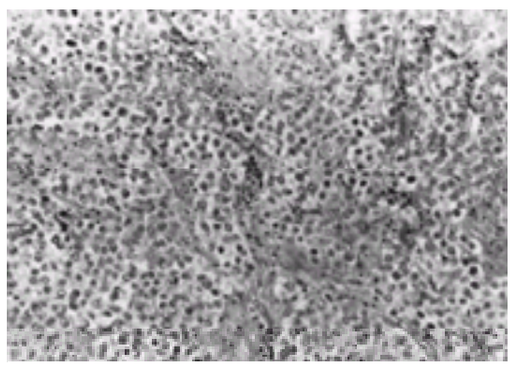

**FIGURE 36-4.** In this germinoma, nests of monomorphic cells with abundant clear cytoplasm and round to oval vesicular nuclei with prominent nucleoli are separated by delicate bands of connective tissue in which a few lymphocytes can be identified.

### Embryonal Carcinoma

Embryonal carcinoma rarely occurs in a pure form in children, being more often a component of a mixed malignant germ cell tumor.[75,77] These carcinomas are characterized by large cells with large, overlapping nuclei and very large, round nucleoli ( Fig. 36-5). The major pattern is epithelial and consists of large nests of cells with varying amounts of central necrosis. Pseudotubular and papillary patterns that may be confused with those of yolk sac tumor are frequent, but the cells are AFP-negative, and the tumors typically lack the eosinophilic hyaline globules characteristic of yolk sac tumors. Unlike other germ cell tumors, embryonal carcinoma is positive for CD30 by immunohistochemical staining.

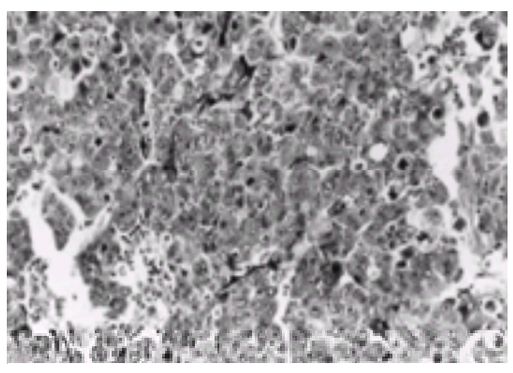

**FIGURE 36-5.** The embryonal carcinoma has large, vesicular, overlapping nuclei with extremely large, prominent nucleoli and moderate amounts of eosinophilic cytoplasm. A number of individually necrotic cells characteristic of this tumor are present.

### Choriocarcinoma

Like embryonal carcinoma, choriocarcinoma rarely occurs outside the context of malignant mixed cell tumors in adolescents.[75,77] The rare case of pure choriocarcinoma detected in infants almost always represents metastasis from maternal or placental gestational trophoblastic primary tumor.[96,97] These tumors characteristically are very hemorrhagic and friable. Microscopically, two types of cells must be present to confirm the diagnosis: *cytotrophoblasts*, which classically

appear as closely packed nests of relatively uniform, medium-sized cells having clear cytoplasm, distinct cell margins, and vesicular nuclei, and *syncytiotrophoblasts*, which represent multinucleate syncytial trophoblastic cells ( Fig. 36-6). The syncytiotrophoblastic elements stain positively for b-HCG, accounting for the associated high concentrations of serum b-HCG in these patients.

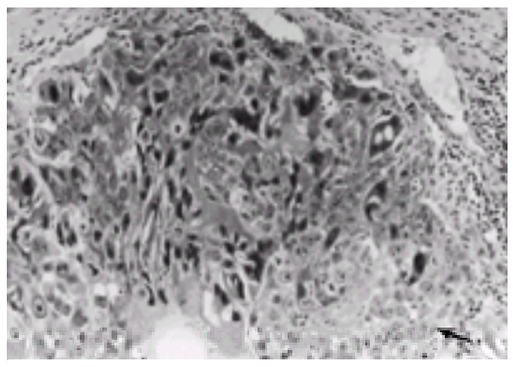

**FIGURE 36-6.** The choriocarcinoma is characterized by nests of small cells (*arrow*) with clear cytoplasm, round vesicular nuclei, and prominent nucleoli (cytotrophoblasts), intimately associated with large, giant syncytiotrophoblasts ( *arrowhead*). Some of these latter cells are multinucleate.

### Gonadoblastoma

Gonadoblastoma is a benign tumor found in dysgenetic gonads of phenotypic female subjects who have at least a portion of the Y chromosome. The tumors usually are small (1 to 3 cm in diameter), soft to firm, gray-tan to brown, and slightly lobulated. They often have a gritty feel on cut sections because of the presence of multifocal calcification. Microscopic features include the proliferation of both germ cells and gonadal sex cord cells. The germ cells show positivity for PLAP. [75] Germinoma frequently develops with gonadoblastoma.[98] In addition, because gonadal dysgenesis may not come to clinical attention until adolescence, the presence of calcifications within a dysgerminoma of an otherwise normal female patient suggests the prior presence of gonadoblastoma and hence merits evaluation of the patient for gonadal dysgenesis.

### Associated Pathologic Findings

#### *Intratubular Germ Cell Neoplasia*

Seminiferous tubules adjacent to testicular malignant germ cell tumors in adolescents and adults may show increased numbers of enlarged, atypical germ cells with abundant clear cytoplasm and prominent nucleoli. These cells show positivity for PLAP and c-kit, similar to the cells of seminomas. [10,99] Such foci have been termed *intratubular germ cell neoplasias* and are thought to represent neoplasia *in situ*.[99,100] and [101] The germ cells in the seminiferous tubules adjacent to infantile testicular malignant germ cell tumors also may be somewhat increased in number and slightly enlarged, with abundant, clear cytoplasm. [102] These cells are negative for the markers of intratubular neoplasia, both PLAP and c-kit, and therefore do not qualify as neoplastic precursor lesions. [10,75]

### Gliomatosis peritonei

A significant number of ovarian teratomas are associated with nodules of mature glial tissue implanted throughout the peritoneum or in lymph nodes. [103,104] Mature glial nodules also have been described in cervical lymph nodes in association with teratomas of the head and neck. [105,106] As long as these tissues are mature and composed only of glial tissue, this process is termed *gliomatosis peritonei*, and neither tumor stage nor prognosis is affected. Unclear is whether implants of immature neural or other mature or immature nonneural tissues are associated with the same benign prognosis.

## CLINICAL MARKERS

The role of clinical markers in the diagnosis of germ cell tumors is well established. In current clinical studies, their usefulness in predicting response or indicating the presence of residual or progressive disease is being examined. [107] These clinically applicable tools in the management of germ cell tumors have been categorized as follows: (a) oncofetoproteins (AFP and b-HCG); (b) cellular enzymes (lactate dehydrogenase [LDH] and PLAP); and (c) cytogenetic and molecular markers (see the section Genetics and Molecular Biology).

### a-Fetoprotein

AFP, an $a_1$-globulin, is the earliest and predominant serum-binding protein in the fetus, reaching its peak concentration at 12 to 14 weeks' gestation and gradually falling to reach an adult normal level of less than 10 ng per dL at approximately age 1 year. [108] As AFP levels begin to decline in fetal development, albumin becomes the principal serum-binding protein. In early embryogenesis, AFP is produced in the yolk sac and later by hepatocytes and the gastrointestinal tract. [108] In 1974, the association between serum elevation of AFP and the natural history of adult germ cell tumors was described. Elevated serum levels or positive immunohistochemical staining of germ cell tumors for AFP indicates the presence of malignant components, specifically yolk sac or embryonal carcinoma. The serum half-life ( $t_{1/2}$) of AFP is 5 to 7 days.[109] Because of the wide variation in levels at birth, especially with infants of less than 40 weeks' gestational age, and the wide variability in $t_{1/2}$ at different ages within the first year of life, [110] difficulties arise in interpreting decay of serum AFP as an indication of residual or recurrent germ cell tumor in infants younger than 8 months.[111,112] Normal ranges have been established to address these problems (Table 36-2).[110,113]

| Age | No. of patients | Mean ± SD (ng/mL) |
|---|---|---|
| Premature | 11 | 134,734 ± 41,446 |
| Newborn | 55 | 48,406 ± 34,718 |
| Newborn–2 wk | 16 | 33,113 ± 32,503 |
| 2 wk–1 mo | 12 | 9,452 ± 12,610 |
| 2 mo | 40 | 323 ± 278 |
| 3 mo | 5 | 88 ± 87 |
| 4 mo | 31 | 74 ± 56 |
| 5 mo | 6 | 46.5 ± 19.0 |
| 6 mo | 9 | 12.5 ± 9.8 |
| 7 mo | 5 | 9.7 ± 7.1 |
| 8 mo | 3 | 8.5 ± 5.5 |

From JT Wu, K Sudar. Serum AFP levels in normal infants. Pediatr Res 1981;15:58, with permission.

**TABLE 36-2. NORMAL RANGES OF SERUM a-FETOPROTEIN IN INFANTS**

Increasing levels of serum AFP, however, are not necessarily indicative of tumor progression. For example, abrupt escalation in serum AFP can occur after chemotherapy-induced tumor lysis.[114] Spurious persistence of elevated serum AFP may reflect an alteration in hepatic function from such conditions as viral hepatitis (hepatitis B, hepatitis C, and human immunodeficiency virus–associated hepatitis), cholestasis secondary to anesthesia, or exposure to phenytoin or methotrexate.[114,115] Marrink et al.[116] reported that measurement of the ratio of concanavalin A to nonbound AFP can be useful in discerning AFP production from tumor

cells (ratio, 12% to 43%) versus production from liver (ratio, <10%). These possible explanations should be considered in interpreting elevations of serum AFP.

Other conditions associated with elevated serum AFP include hepatoblastoma, pancreatic and gastrointestinal malignancies, lung cancers, and benign liver conditions, including hepatic dysfunction and cirrhosis. [117,118]

### b-Subunit of Human Chorionic Gonadotropin

Human chorionic gonadotropin is a glycoprotein comprised of a- and b-peptide subunits and normally is synthesized during pregnancy by syncytiotrophoblasts of the placenta to maintain viability of the corpus luteum. The a-subunit is similar to a-peptides of other hormones, such as luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone; the b-subunit is antigenically distinct, serving as the basis for the method of serum assay. [119] Minute amounts, less than 5 mIU/mL, are detected in serum of healthy adults.[120] The serum $t_{1/2}$ of b-HCG is 24 to 36 hours.[120]

Elevation of serum b-HCG in patients with germ cell tumors implies the presence of clones of syncytiotrophoblasts, such as choriocarcinoma, or of syncytiotrophoblastic giant cells, found frequently in germinomas (pure seminomas or dysgerminomas) and occasionally in adult embryonal carcinoma. Immunoperoxidase staining of tumor for b-HCG detects these hormone-containing elements.[121]

Like serum AFP, sudden elevation of serum b-HCG occurs after cell lysis secondary to chemotherapy. [114] Iatrogenic hypogonadism secondary to bilateral orchiectomy, oophorectomy, or chemotherapy also may be associated with rising levels of serum b-HCG because of an increase in luteinizing hormone that results in immunologic cross-reactivity.[115] Other conditions in which modest elevations of serum b-HCG have been reported include multiple myeloma and other malignancies of liver, pancreas, gastrointestinal tract, breast, lung, and bladder. [117] Simultaneous elevation of serum AFP and b-HCG has been described in ovarian embryonal carcinoma in an 11-year-old and in patients with polyembryoma.[122]

### Other Markers

Because some germ cell tumors with identifiable malignant elements do not produce measurable amounts of serum AFP or b-HCG, other markers with potential prognostic value have been investigated. Serum LDH, a glycolytic enzyme that appears to correlate with growth and regression of various solid neoplasms, has not shown specificity for a specific histologic subtype of germ cell tumors. In patients with dysgerminoma, serum levels of the LDH isoenzyme 1, the gene for which resides on 12p,[120] correlate with the tumor burden and aid in the planning and assessment of surgical management. [123] PLAP is a fetal isoenzyme of alkaline phosphatase that is elevated in the sera of up to 30% of patients with stage I disease and of almost 100% of cases with advanced seminoma. [124,125] As with AFP and b-HCG, immunohistochemical staining for PLAP sometimes is useful in determining the origin of histologically undifferentiated tumors. [120]

Although elevated serum levels of carcinoembryonic antigen are reported in patients with ovarian tumors, the usefulness of this antigen has been hampered by lack of tumor specificity and correlation to disease natural history. [126] The carbohydrate antigen CA-125, which is related to the tissues of the coelomic epithelium and müllerian ducts, has been assessed in ovarian cancers of germ cell and epithelial origin. CA-125 has been reported to have some correlation with other tumor markers and to be of value in monitoring patients with ovarian tumors of germ cell, epithelial, and stromal origin, [127,128] although its utility in these patients remains to be defined because of the limited numbers of patients studied to date. [126] Extreme elevation of serum levels of CA-19-9, an antigen in the Lewis A blood group system, has been reported at recurrence of ovarian immature and mature teratoma with yolk sac elements [129]; however, the role of CA-19-9 is even less clear than that of CA-125.[126]

## TREATMENT OVERVIEW

The heterogeneity of pediatric germ cell tumors relative to histologic type, site of origin, age, and stage demands an individualized, multimodality treatment plan. In recent years, the timing and aggressiveness of surgery have been refined with the development of a variety of effective chemotherapeutic agents. The role of radiotherapy is yet to be established completely in the treatment of pediatric germ cell tumors.

### Principles of Surgery

Surgical resection is the therapy of choice in benign tumors, such as teratomas. With malignant lesions, removal is indicated, if possible. However, given the availability of effective chemotherapy, resection should not be undertaken to the point of sacrificing vital structures. In this situation, only debulking or biopsy is appropriate. After initial chemotherapy, second-look surgery serves to assist in achieving complete response in selected patients. Surgical considerations that are unique to the various germ cell tumors are addressed in the following sections.

### Principles of Chemotherapy for Germ Cell Tumors

In the last two decades, substantial improvements in the cure rates for pediatric germ cell tumors have occurred, stemming in large part from the evolution of effective chemotherapeutic strategies, most of which were developed for and investigated in the larger adult population with these neoplasms. Although available data from children are more limited, the adult experience appears to hold true for most childhood germ cell tumors as well. In both populations, the advent of effective combination chemotherapy has attenuated the natural history differences noted in germ cell tumors of different histopathologic subtypes and sites of origin.

As single agents, actinomycin-D, vinblastine, bleomycin, doxorubicin, cisplatin, and etoposide have proved efficacious in treating various tumors of germ cell origin, with objective response rates (complete plus partial responses) ranging from 28% to 100%. [130,131,132,133,134] and [135] Combinations of these agents demonstrating synergistic activity have served as the basis for numerous multidrug regimens ( Table 36-3).

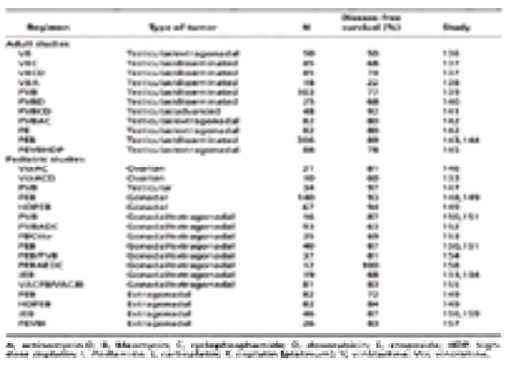

**TABLE 36-3. EVOLUTION OF COMBINATION CHEMOTHERAPY IN GERM CELL TUMORS**

Most adult studies have been conducted in patients with testicular and extragonadal tumors, primarily with advanced or disseminated disease. Before platinum, disease-free survival (DFS) ranged from 22% to 74%, with steady increases related to the addition of agents to the vinblastine-bleomycin backbone. [136,137] and [138] The advent of cisplatin and its incorporation into combination regimens resulted in a substantial increase in DFS, to between 68% and 92%. [139,140,141,142,143,144] and [145] Use of the platinum-based combinations may obviate the need for four- and five-agent regimens and their associated side effects. Bosl et al. [142] compared a regimen of vinblastine, bleomycin, cyclophosphamide, cisplatin, and actinomycin-D with one of cisplatin plus etoposide and reported almost identical rates of complete response (96% vs. 93%, respectively) and DFS (80% in both groups). Moreover, the two-drug regimen was associated with far less toxicity. Einhorn and Williams [140] could not demonstrate additional benefit after the addition of doxorubicin to a regimen of cisplatin (platinum), vinblastine, and bleomycin (PVB) in the treatment of disseminated testicular cancer in adults and suggested that, with the improvement in outcome in these patients, the goal of future trials should be to reduce toxicity. In a randomized

study of 184 patients with favorable-prognosis testicular tumors comparing outcome after three versus four courses of PVB, the rates of complete response and survival were almost identical, and less toxicity was observed in the short-course arm.[144] Administration of cisplatin in higher doses (40 mg per $m^2$ per day × 5 as compared with 20 mg per $m^2$ per day × 5) with saline and mannitol diuresis has proved superior to conventional-dose cisplatin and appears to overcome platinum resistance.[145] Although more toxicity is associated with the higher doses of platinum, toxicity still is tolerable.

The pediatric record, although not as extensive, has mirrored the adult experience in that combination chemotherapy has been found to be superior to single or dual agents and the addition of platinum has further boosted the efficacy of these regimens.[133,134,146,147,148,149,150,151,152,153,154,155,156,157] and [158] The dosages and methods of administration of current regimens employed in pediatric germ cell tumors [PVB; cisplatin (platinum), etoposide, and bleomycin (PEB); and carboplatin, etoposide, and bleomycin (JEB)] are given in Table 36-4. PEB was the standard treatment used in intergroup studies by the Pediatric Oncology Group (POG) and the Children's Cancer Group (CCG). For high-risk germ cell tumors, PEB was compared to a combination of high-dose cisplatin plus etoposide and bleomycin.[148,149] The preliminary experience of Pinkerton et al.[159] in the United Kingdom with carboplatin in pediatric germ cell tumors provides the basis for future studies comparing regimens containing cisplatin or carboplatin with the goal of reducing permanent toxicity. In a more recent report from the United Kingdom Children's Cancer Study Group's Germ Cell Tumour Studies, carboplatin (600 mg per $M^2$) with etoposide and bleomycin (JEB) demonstrated efficacy over standard-dose cisplatin [100 mg per $M^2$ (PEB)]. These data must be interpreted with caution, as comparisons were not made with high-dose cisplatin, the study was retrospective, and the standard platinum group was made up of small patient numbers (N = 21).[156] Marrow-ablative doses of carboplatin and etoposide followed by autologous marrow reinfusion may provide a method of salvaging patients who experience relapse or whose disease proves refractory to treatment.[160]

| Regimen | Components | Administration | Study |
|---|---|---|---|
| PVB | Cisplatin [platinum] | 20 mg/m² i.v. days 1–5 | 139 |
| | Vinblastine | 0.2 mg/kg i.v. days 1 & 2 | |
| | Bleomycin | 15 U/m² i.v. days 2, 9, 16 | |
| PEB | Cisplatin [platinum] | 100 mg/m² i.v. day 1 | 222 |
| | Etoposide | 120 mg/m² i.v. days 1–3 | |
| | Bleomycin | 15 U/m² i.v. day 2 | |
| JEB | Carboplatin | 600 mg/m² i.v. day 1 | 159 |
| | Etoposide | 120 mg/m² i.v. days 1–3 | |
| | Bleomycin | 15 U/m² i.v. day 2 | |

B, bleomycin; E, etoposide; J, carboplatin; P, cisplatin; V, vinblastine.

**TABLE 36-4. CHEMOTHERAPEUTIC REGIMENS FOR PEDIATRIC GERM CELL TUMORS**

Specific recommendations for incorporating chemotherapy into the management of pediatric germ cell tumors are discussed separately for each tumor. Generally, in low-risk disease (stage I testicular and ovarian tumors), no chemotherapy is indicated if careful observation is possible. Patients with moderate-risk gonadal tumors or progression of disease in untreated tumors may be managed adequately with three to four courses of a platinum-containing regimen. For higher-risk patients (higher-stage testicular or ovarian tumors and extragonadal tumors), 6 months of a platinum-based chemotherapeutic regimen is indicated.

## OVARIAN TUMORS

Ovarian tumors are rare, accounting for only some 1% of childhood malignancies.[161,162] Although ovarian tumors may occur at any age, the incidence increases at 8 to 9 years and peaks at 19 years.[161] For children younger than 15 years, these tumors are most common between the ages of 10 and 14.[163,164] As it does with testicular tumors, the frequency of ovarian germ cell tumors parallels gonadotropin release, implicating hormonal factors in their etiology.[163,165,166] In contrast to adult ovarian tumors, two-thirds of pediatric ovarian tumors are of germ cell origin, with tumors of epithelial and stromal origin occurring less frequently.[161,164,167,168] The types of malignant ovarian germ cell tumors are detailed in Table 36-1. In order of decreasing frequency, the categories are dysgerminoma, endodermal sinus tumor (yolk sac carcinoma), immature teratoma, mixed germ cell tumor, and embryonal carcinoma.[169]

Abdominal pain is the presenting symptom in up to 80% of patients.[163,164,170] The pain can be chronic in nature but, in up to one-third of patients, mimics an acute abdomen.[163] Most of these latter patients have associated ovarian torsion[163,164] and often undergo exploratory surgery for presumed acute appendicitis.[163,164] Other presenting signs and symptoms include a palpable abdominal mass, fever, constipation, amenorrhea, vaginal bleeding and, rarely, urinary frequency and dysuria.[164,171] Precocious puberty, more often associated with ovarian stromal tumors, has been described in endodermal sinus tumor, choriocarcinoma, and mixed teratoma with sarcomatous and non–germ cell carcinomatous elements.[171]

Ultrasonography is used most often for the initial evaluation of patients with abdominal or pelvic masses and will differentiate cystic from solid masses.[172,173] Although the presence of a solid ovarian mass raises the suspicion of malignancy, the majority will be benign teratoma.[163] Even the use of color and Doppler ultrasonography techniques will not distinguish benign from malignant lesions.[174] Computed tomography (CT) of the abdomen and pelvis is helpful in identifying the site of origin, the extent of tumor, the presence of calcifications or fat, and metastatic disease. In a pediatric study evaluating the use of CT for patients with ovarian masses, Jabra et al.[175] noted that only 55% of children with teratomas had radiologic evidence of fat as compared to 94% of adult patients in similar circumstances.[176] CT appears useful also in monitoring tumor response and, as reported by Moskovic et al.,[177] may detect features suggestive of transformation of malignant disease to benign disease after chemotherapy (retroconversion). In that report, tumor maturation, characterized radiographically by increased density, more circumscribed margins, and the presence of internal calcification with fatty areas and cystic changes, was observed and followed up in seven patients with immature teratomas. At second-look surgery, all seven patients had mature teratoma without malignant elements and remained well from 1 to 6 years after treatment.

Because patients can develop metastatic disease to the thoracic lymph nodes, lung and, rarely, bone,[169,178,179,180] and [181] staging evaluation should include chest CT and $^{99m}$TC-pertechnetate–enhanced scintigraphy. Assessment of serum tumor markers AFP and b-HCG is essential, as the majority of pediatric patients with ovarian germ cell tumors have an endodermal sinus tumor component.[150,182]

The staging system designed by the International Federation of Gynecology and Obstetrics and shown in Table 36-5 creates a framework for staging pediatric tumors.[183] Based on clinical, surgical, and pathologic findings, this system includes cytologic examination of any thoracic or peritoneal fluid. A modification of this staging system (Table 36-6) was devised by the POG and the CCG for use in intergroup studies. This surgicopathologic system refines the International Federation of Gynecology and Obstetrics system by accounting for (a) the higher risk of tumor recurrence in patients who have positive peritoneal washing and are therefore up-staged, (b) the utility of tumor markers for prediction of outcome, and (c) the lack of negative prognostic impact of gliomatosis peritonei if only mature glial tissue is present. The clinical features of the various ovarian tumors are summarized in Table 36-7 and detailed here.

| Stage | Extent of disease |
|---|---|
| I | Tumor limited to the ovaries |
| Ia | Limited to one ovary; no ascites. No tumor on external surface; capsule intact |
| Ib | Limited to both ovaries; no ascites. No tumor on external surfaces; capsule intact |
| Ic | Limited to one or both ovaries but with tumor on surface of one or both ovaries; or with capsule ruptured; or with positive ascites or positive peritoneal washings |
| II | Tumor involving one or both ovaries with pelvic extension |
| IIa | Extension and/or metastases to uterus and/or tubes only |
| IIb | Extension to other pelvic tissues |
| IIc | As in IIa or IIb but with positive ascites or positive peritoneal washings; or with capsule ruptured |
| III | Tumor involving one or both ovaries with peritoneal implants outside the pelvis and/or positive retroperitoneal or inguinal lymph nodes; extension to small bowel or omentum; superficial liver metastases |
| IIIa | Limited to true pelvis grossly with negative nodes but histologically confirmed microscopic seeding of abdominal peritoneal surfaces |
| IIIb | Limited to one or both ovaries with negative nodes but histologically confirmed implants of abdominal peritoneal surfaces, none >2 cm diameter |
| IIIc | Abdominal implants >2 cm diameter and/or positive retroperitoneal or inguinal nodes |
| IV | Tumor of one or both ovaries with distant metastases outside of peritoneal cavity; parenchyma liver metastases; pleural effusion, if present, must have positive cytology |

From III, Sommer, Dissection and staging of malignant tumors in the female pelvis. Int J Gynaec Obstet 1971;9:312, with permission.

**TABLE 36-5. INTERNATIONAL FEDERATION OF GYNECOLOGY AND OBSTETRICS STAGING FOR OVARIAN TUMORS**

| Stage | Extent of disease |
|---|---|
| I | Limited to ovary or ovaries; peritoneal washings negative for malignant cells<br>No clinical, radiographic, or histologic evidence of disease beyond the ovaries<br>Tumor markers normal after appropriate postsurgical half-life decline<br>The presence of gliomatosis peritonei[a] does not up-stage patient |
| II | Microscopic residual or positive lymph nodes (≤2 cm as measured by pathologist)<br>Peritoneal washings negative for malignant cells<br>Tumor markers positive or negative<br>The presence of gliomatosis peritonei[a] does up-stage patient |
| III | Lymph node with malignant metastatic nodule (>2 cm as measured by pathologist)<br>Gross residual or biopsy only<br>Contiguous visceral involvement (omentum, intestine, bladder)<br>Peritoneal washings positive for malignant cells<br>Tumor markers positive or negative |
| IV | Distant metastases, including liver |

[a]Peritoneal nodules composed entirely of mature glial tissue and having no malignant elements.

**TABLE 36-6. CHILDREN'S ONCOLOGY GROUP STAGING OF OVARIAN GERM CELL TUMORS**

| Tumor type | Median age (yr) | Relative frequency (%) | Features |
|---|---|---|---|
| Dysgerminoma | 15 | 34 | Rapidly developing; 14–25% with other germ cell elements; very radiosensitive |
| Endodermal sinus tumor (yolk sac) | 18 | 16 | ↑AFP; 75% stage I; all patients require chemotherapy because of high risk for relapse, even in low-stage disease |
| Teratoma | | | |
| Mature (solid, cystic) | 10–15 | 33 | Neuroglial implants may occur with cystic or solid teratomas but do not affect prognosis; surgery is mainstay of treatment |
| Immature | 11–14 | 10 | Grading system based on amount of neuroepithelium present; prognosis inversely related to stage and grade; 35% with ↑AFP |
| Embryonal carcinoma | 14 | 6 | 47% prepubertal; ↑HCG and precocious puberty common; chemotherapy indicated |
| Mixed malignant germ cell tumor | 16 | 11 | 40% premenarchal; 30% sexually precocious; AFP/HCG may be increased |
| Gonadoblastoma | 8–18 | 1 | Associated with dysgenetic gonads and sexual maldevelopment; removal of both gonads is treatment of choice |
| Other (polyembryoma, choriocarcinoma) | NA | <1 | Rare in children |

AFP, α-fetoprotein; HCG, human chorionic gonadotropin; NA, not available; ↑, increased.

**TABLE 36-7. CLINICAL FEATURES OF PEDIATRIC OVARIAN TUMORS**

### Teratoma

Teratomas are composed of well-differentiated tissues arising from the three germ cell layers (endoderm, ectoderm, and mesoderm) and usually contain tissues foreign to the anatomic site of origin.[184] Teratomas occasionally can be monodermal or composed of a single germ cell layer, which typically includes thyroid, carcinoid, or neuroectodermal tissue.[184,185] On the basis of their imaging findings, teratomas can be classified as either cystic or solid. Teratomas can be classified also according to their histologic composition as follows: mature, containing well-differentiated tissues; immature, containing varying degrees of immature fetal tissue, most often neuroectodermal; or malignant, containing at least one of the malignant germ cell elements. The management of mature and immature teratomas is based on the most malignant element present. Recent reports suggest that monodermal teratomas composed of neuroectodermal tissue do not respond as well to germ cell tumor–directed therapy.[186,187]

The mature cystic teratoma is the most common type of germ cell tumor and, like all ovarian tumors, is most common during the second decade of life. Approximately 10% of patients with teratomas have bilateral tumors[185] and, in this instance, every effort should be made to preserve fertility.[188] Although rare, between 1% and 3% of mature cystic teratomas present with ovarian torsion, and an additional 1% to 3% can rupture spontaneously, which can be fatal.[185,189] Rupture of the teratoma may be associated with two clinical pictures: acute peritonitis (sudden rupture of contents) or a granulomatous peritonitis, resulting from a chronically leaking tumor, which typically presents with multiple small peritoneal implants and adhesions.[189] The solid teratoma also is most frequently benign, although it can be associated with peritoneal implants and lymphatic spread.[190,191] The treatment of choice for patients with mature teratomas is surgical resection.

Immature teratomas are pathologically distinct from the benign and malignant teratomas. As discussed in the section Pathology, they are graded (from 1 to 3) by the amount of immature tissue present, using the system of Norris et al.[192] and later modified by Robboy and Scully.[193] In adult series, including some pediatric patients, the median age at diagnosis is between 17 and 21 years.[192,194,195,196,197 and 198] However, a recent intergroup pediatric study of completely resected ovarian immature teratomas reported a median age of 10 years.[199] Thus, up to 50% of pediatric patients with ovarian immature teratomas might be premenarcheal. As with other ovarian tumors, the most frequent complaint is abdominal pain, occurring in 75% to 95% of patients,[192,195,197] and a palpable abdominal mass in 44% to 88%.[192,195] At laparotomy, the majority of tumors are unilateral, but spread beyond the ovary is documented in from 31% to 50%.[192,195,197] Common metastatic sites include lymph nodes, liver, peritoneal surfaces and, rarely, the lung.[192,195]

Much controversy surrounds the management of immature teratomas in children. Norris et al.[192] described 58 patients with ovarian immature teratomas and reported that 70% of those with grade 3 tumors underwent relapse. On the basis of this experience, the recommendations for treatment of ovarian immature teratomas have been to proceed with chemotherapy in patients with grades 2 and 3 tumors.[194,195,196,197 and 198] In contrast, a similar relationship of histologic grade to outcome has not been reported in pediatric patients with testicular and extragonadal immature teratomas.[200,201,202,203 and 204] Thus, some have suggested that the clinical behavior of immature teratomas in the pediatric population might be different from their adult counterparts.[203,204] This hypothesis was tested in a prospective study conducted by the POG and the CCG. In that intergroup trial, the 44 patients with ovarian immature teratomas were treated with complete surgical resection followed with observation only.[199] The 4-year event-free survival of this subset was 98%, with only one patient developing recurrent tumor. On central pathologic review, 30% of these patients had microscopic foci of malignant elements, which did not appear to affect outcome adversely. As in other studies,[195] approximately 30% of patients had elevated serum AFP, but in not all of those were microscopic foci of endodermal sinus tumor detected.[199]

The prognostic significance of mature peritoneal implants (gliomatosis peritonei)[193,205] in patients with immature teratomas is uncertain. Although having peritoneal implants does not up-stage the patient's disease, reports have cited malignant transformation of these elements[206] and growth during and after chemotherapy.[177,207,208]

The experience with immature teratomas in the recent intergroup study from the POG and the CCG[187,199] strongly supports using complete resection only, followed by close observation of serum tumor markers and diagnostic imaging. Those patients unable to undergo complete resection should be offered chemotherapy similar to that used for other germ cell tumors (i.e., PEB). Although ample adult data report using these regimens,3[194,195 and 196,198] the pediatric experience is limited to a recent German trial in which the recurrence rate approached 18% and appeared related to the degree of resection.[209]

### Dysgerminoma

Dysgerminoma represents the female counterpart of testicular seminoma and is the most common ovarian germ cell tumor of childhood and adolescence.[210,211 and 212] Although it can occur at any age during childhood, it occurs most frequently during adolescence, with a peak age at 19 years.[166] The most common presenting signs and symptoms include the development of an abdominal mass in 50% of patients[181,213] and abdominal pain in 36% to 48%.[181,213,214] The majority of patients present with stage I disease,[181,213,214 and 215] but the tumor can spread via the lymphatic system to the left kidney and para-aortic region, and it can disseminate to the liver, lung, or supradiaphragmatic lymph nodes.[181] Dysgerminoma can be bilateral in up to 20% of cases.[212,215] When dysgerminoma occurs as a component of a mixed germ cell

tumor, therapy should be directed against the most malignant element present.

Unlike other ovarian germ cell tumors, dysgerminomas were curable even before the advent of effective chemotherapy, with survival rates ranging from 86% to 94% after surgery and radiotherapy.[181,212,213 and 214] Although radiotherapy produces very high cure rates, it has significant late sequelae that can be particularly severe in children who have not achieved their final adult height.[214,216] In addition, because the majority of patients with dysgerminoma present during their reproductive years and the use of radiotherapy has the potential to cause infertility, an increasing emphasis has been placed on attempts to treat patients with chemotherapy instead of radiotherapy. The adult experience with this strategy confirms the chemosensitivity of this tumor.[217,218 and 219] On the basis of these data, the present recommendations are that patients with stage I dysgerminomas be observed after surgical resection. However, some authors would recommend the use of cisplatin-based therapy even in stage I patients, to reduce the 20% risk of recurrent disease.[217] Patients with more advanced disease will require four to six cycles of cisplatin-based therapy for cure.[217,219] Following this approach, sustained remissions in 90% of patients can be anticipated,[219,220] and up to 70% of patients will have normal menstrual function.[210]

### Yolk Sac Tumor

Yolk sac tumor, also known as *endodermal sinus tumor*, is the most aggressive of the ovarian malignant germ cell tumors[180,221] and the most common ovarian malignant germ cell tumor in pediatric patients.[150,152,182,222] The median age at the time of diagnosis is approximately 19 years,[180,221] and most patients have elevated levels of AFP.[150,182] The tumor can spread to lymphatics and peritoneal structures quickly, accounting for the acute onset of symptoms.[180,221] Abdominal pain occurs in up to 80% of patients; 75% of patients have abdominal masses.[180,221] Distant metastases are seen in liver, lungs, lymph nodes and, rarely, in bone.[180] Historically, the prognosis for these tumors was poor with surgery and radiotherapy.[180,221] With the advent of cisplatin-based therapy, outcomes have improved dramatically, and DFS for all stages presently exceeds 80%.[143,148,151,155,217,223]

### Embryonal Carcinoma

In contrast to adult testicular tumors, embryonal carcinoma is a rare component of ovarian germ cell tumors.[184] The median age at diagnosis is 14 years, and the most common presentation is an abdominal mass (80%) or abdominal pain (53%) or both.[180] Because the tumor has multinucleate giant cells similar to those in syncytiotrophoblasts, which can produce HCG, patients often present with precocious puberty, amenorrhea, or hirsutism.[180] Although morphologically similar to endodermal sinus tumor, embryonal carcinoma can be readily distinguished pathologically. Approximately one-half of the patients present with stage I tumors and undergo unilateral salpingo-oophorectomy only. However, the tumor may extend into peritoneal surfaces or metastasize to lymph nodes, lung, and liver.[180] After surgical resection, survival is only 50%, suggesting the presence of micrometastases. As this tumor is rare, the data regarding the impact of chemotherapy on natural history are scant. However, on the basis of the adult experience with testicular tumors,[224] the recommended treatment course is cisplatin-based therapy.[152,182]

### Mixed Malignant Germ Cell Tumor

Mixed malignant germ cell tumors comprise a subset of germ cell tumors containing more than one malignant component. Various reports suggest that 10% to 40% of patients with malignant germ cell tumors have mixed histology.[150,152,169,182,211] The median age at diagnosis is 16 years,[169,211] and 40% of patients are prepubertal.[169] Most patients present with abdominal pain or an abdominal mass (or both).[169,211] Approximately 30% of prepubertal patients have precocious puberty.[169]

The most common histologic component is dysgerminoma, but immature teratoma, endodermal sinus tumor, and embryonal carcinoma also can be detected in varying proportions. As with the other germ cell tumors, evaluation of tumor markers at the time of diagnosis is essential, as patients with mixed germ cell tumors having endodermal sinus tumor, embryonal carcinoma, or dysgerminoma may have elevated markers that are useful for diagnosis and follow-up. In general, these patients are managed on the basis of the most malignant element present in the specimen, usually with the use of cisplatin-based chemotherapy.

### Choriocarcinoma

Pure ovarian choriocarcinomas are rare and most commonly are components of mixed ovarian germ cell tumors.[184,225,226] The tumor typically produces HCG that can produce false-positive pregnancy test results and, in prepubertal patients, precocious puberty.[225] Choriocarcinomas can be either gestational or nongestational, but the diagnosis of nongestational choriocarcinoma is difficult in women of childbearing age and can be confirmed only in prepubertal patients.[225,226 and 227]

Choriocarcinoma is an aggressive subtype of germ cell tumor with a propensity for early metastases to lung, liver, and brain. Gestational choriocarcinomas are exquisitely sensitive to methotrexate[228] and are highly curable even in the presence of widespread metastases.[229,230,231 and 232] In contrast, the small number of patients analyzed with nongestational choriocarcinomas may have a less favorable prognosis.[226,227,233] Such patients usually are managed as for other ovarian germ cell tumors.[233]

### Polyembryoma

Polyembryomas are rare tumors of the ovary, often reported in combination with other neoplastic components.[234,235] Their presenting signs and symptoms are similar to those of other ovarian germ cell tumors. A report has cited precocious puberty secondary to HCG production by this tumor.[236] Polyembryomas are very malignant tumors and, although not radiosensitive, have been reported to respond to chemotherapy similar to that used for other malignant germ cell tumors of the ovary.[236]

### Gonadoblastoma

Gonadoblastomas are tumors composed of germ cells intermixed with stromal cells (usually Sertoli or granulosa cells with or without Leydig cells).[237,238 and 239] These neoplasms develop during the teenage years, most frequently in patients with XY gonadal dysgenesis, although a small number might occur in patients with 45 XO/46, –XY mosaicism.[238,239 and 240] Most often, such patients present to their physicians for evaluation of amenorrhea,[238] eading to chromosomal analysis. Patients may have a eunuchoid body habitus, lack of secondary sexual characteristics, elevated gonadotropin levels, and the presence of streak gonads.[238,241,242] Because of the 30% risk for malignancy,[239] the management of patients with this condition should include prophylactic removal of their streak gonads.[238,239]

Most gonadoblastomas are small to medium-sized and behave in a benign fashion unless overgrowth of a malignant germ cell element is present. Although most tumors are unilateral, up to 36% are bilateral. Dysgerminoma is the most common malignant element occurring in approximately 50% of cases.[237,239] Although data are limited, chemotherapy used for other malignant germ cell tumors should be considered, depending on the malignant germ cell tumor element present and the stage of the disease.[243]

## TESTICULAR TUMORS

Pediatric testicular tumors are rare, accounting for 2% of solid malignant neoplasms in boys.[201,244] The annual incidence in the United States for white and black boys younger than 15 years of age is 1.1 and 0.9 per 100,000 population, respectively.[1] Although adolescent tumors resemble those seen in adults, prepubertal tumors differ with respect to incidence, clinical manifestations, histopathology, and prognosis.[201,244] The major risk factor for development of testicular tumors during childhood is the presence of an undescended testicle[245,246] (see the section Cryptorchid Testis).

Approximately 75% of childhood testicular tumors are of germ cell origin (Table 36-8), as compared with more than 90% of those found in the adult population.[201,247] Two-thirds of the germ cell tumors are endodermal sinus tumors, and a smaller portion are teratomas. Rarely, a mixture of germ cell and stromal components (gonadoblastoma) is noted in a phenotypic female patient with dysgenetic gonads and male karyotype.

| Tumor type | Median age (yr) | Relative frequency (%) | Features |
|---|---|---|---|
| Endodermal sinus tumor (yolk sac) | 2 | 26 | Most common of malignant germ cell tumors of the testes; ↑ AFP; as compared to adult cases, pediatric tumors are pure histologically. 85% stage I. Chemotherapy reserved for higher stage or recurrent disease |
| Teratoma | 3 | 24 | Poorly differentiated histologic features do not impart a malignant course in children. Surgery alone usually is sufficient treatment. |
| Embryonal carcinoma | Late teens | 20 | Uncommon in young children; ↑AFP a HCG; managed as for adults, with retroperitoneal lymphadenectomy ± chemotherapy ± irradiation based on stage. |
| Teratocarcinoma | Late teens | 13 | 80% stage I with 75% survival after surgery alone. More advanced disease requires multimodality therapy. |
| Gonadoblastoma | 5–10 | <1 | Associated with sexual maldevelopment syndromes, bilateral involvement in 30%. Bilateral removal of gonads is treatment of choice. |
| Others (seminoma, mixed germ cell tumor, choriocarcinoma) | NA | 16 | Rare in children. |

AFP, α-fetoprotein; HCG, human chorionic gonadotropin; NA, not applicable; ↑, increased.

**TABLE 36-8. CLINICAL FEATURES OF PEDIATRIC TESTICULAR TUMORS**

Almost all testicular tumors are identified as irregular, nontender scrotal masses. [248] The paucity of associated signs or symptoms may lead to delays in evaluation for up to 6 months for germ cell tumors and 24 months for non–germ cell tumors.[201] Although testicular tumors do not transilluminate, 20% may be associated with reactive hydroceles at the time of diagnosis. [249,250] Li and Fraumeni[251] reported a 21% incidence of concomitant inguinal hernias. Approximately 90% of pediatric tumors are localized, as compared with 39% in adults. [250] Metastatic disease typically spreads to the lymph nodes of the retroperitoneum and chest.

Preoperative assessment of serum markers (e.g., AFP, b-HCG) is essential because it serves as the basis for staging and patient monitoring. Ultrasonography is instrumental in localizing the scrotal mass with respect to the testicle and for distinguishing a simple hydrocele from a reactive hydrocele associated with the testicular tumor. Metastatic evaluation should include ultrasonography and CT of the abdomen and pelvis, in addition to chest radiography and CT and bone scintigraphy with $^{99m}$Tc-pertechnetate. Because focal atelectasis after general anesthesia can mimic metastatic pulmonary lesions, CT scan of the chest before surgery is recommended. Lymphangiography no longer is an acceptable method of evaluating the retroperitoneum in children. [244,252]

Previously, the only staging system used consistently for prepubertal patients with testicular tumors was that proposed by Evans et al., [253] which defined disease according to whether it was confined to scrotum, metastatic to regional lymph nodes (within the abdomen), or metastatic to distant sites. Recently, investigators from the POG and the CCG developed staging criteria (Table 36-9) that also account for tumor marker status and less-than-ideal transscrotal surgery to these tumors. Features unique to the various histologic subtypes of testicular tumors are summarized in Table 36-8 and are detailed later.

| Stage | Extent of disease |
|---|---|
| I | Limited to testes<br>Completely resected by high inguinal orchiectomy or transscrotal orchiectomy with no spill<br>No clinical, radiographic, or histologic evidence of disease beyond the testes<br>Tumor markers normal after appropriate postsurgical half-life decline; patients with normal or unknown markers at diagnosis must have a negative ipsilateral retroperitoneal node dissection to confirm stage I |
| II | Trans-scrotal orchiectomy with gross spill of tumor<br>Microscopic disease in scrotum or high in spermatic cord (<5 cm from proximal end)<br>Retroperitoneal lymph node involvement (≤2 cm)<br>Increased tumor marker after appropriate half-life |
| III | Retroperitoneal lymph node involvement (>2 cm)<br>No visceral or extra-abdominal involvement |
| IV | Distant metastases, including liver |

**TABLE 36-9. PEDIATRIC ONCOLOGY GROUP/ CHILDREN'S CANCER GROUP STAGING OF TESTICULAR TUMORS**

The classic surgical approach for both diagnosis and treatment of testicular tumors is radical inguinal orchiectomy, with *en bloc* excision of spermatic cord structures and testicle. In adults, Giguere et al.[254] reported recurrence of tumor in inguinal and retroperitoneal sites in association with surgical procedures that violate the scrotum, tunica vaginalis, and tunica albuginea. This adverse outcome was attenuated by subsequent radical orchiectomy with partial scrotectomy, which excised the previous incision site, combined with retroperitoneal node dissection and chemotherapy. The low risk in pediatric tumors, assuming the transscrotal approach is not associated with tumor spillage, and the excellent response of pediatric tumors to modern chemotherapy obviate the need for up-staging disease in these otherwise low-risk patients or for performing a modified retroperitoneal lymphadenectomy in this situation.

### Cryptorchid Testis

The cryptorchid testicle is the most significant risk factor for the development of testicular carcinoma. Considering that 10% of patients with testicular cancers are found to have undescended testicles and that the prevalence of undescended testicle is estimated at 0.23%, the theoretic risk for testicular cancer has been estimated to be 10- to 50-fold higher in boys and men with undescended testicles. [245,255,256]

Histologic abnormalities of germinal, tubular, or Sertoli tissue occur in 85% of undescended testicles, although only a few (fewer than 1%) are truly dysgenetic. [256,257] Because surgical relocation of the testes decreases the incidence of histologic anomalies, extrascrotal location appears more important than pathologic factors regarding malignant potential. The specific locale of the cryptorchid testicle does influence the risk of malignancy: The 8% to 22% of undescended testicles situated in the abdomen[255,257,258] account for approximately 45% of malignancies.[258,259] and [260] Typically, the histologic tumor types related to the cryptorchid testicle are seminoma and embryonal carcinoma, and presentation is typically in the fourth decade of life. [101,245,257]

Hormonal therapy with HCG has been reported to induce descent of the testicle into the scrotum in 15% of patients aged 2 to 5 years and in 44% aged 10 to 14 years. [261] Hadziselimovic[262] noted that 60% of cases responded to gonadotropin-releasing hormone (GnRH) but did not account for retractile testes in the study. In a double-blind randomized study of GnRH by nasal insufflation in which retractile testes were excluded, GnRH performed no better than placebo. [263]

Orchidopexy is advised on the undescended testicle after 6 months and before 18 months. [256] This earlier intervention, however, may not prevent the subsequent development of testicular carcinoma.[255,256] Although extensive data from children undergoing orchidopexy before age 5 are unavailable, reduction in the risk of cancer with decreasing age of orchidopexy has been reported. [264] The prepubescent child with an intra-abdominal testicle undergoing orchidopexy should have a testicular biopsy performed in search of carcinoma *in situ*. The pubertal male patient with an undescended testicle should undergo inguinal exploration and orchiectomy if a normally placed contralateral testicle is present. In the absence of a contralateral testicle, orchidopexy may be performed, but a biopsy of the testicle is mandatory, as is close follow-up. Parents and older patients should be advised of the importance of extended periodic medical examination and frequent self-examination throughout such a patient's life.

### Yolk Sac Tumor

Yolk sac tumor is the malignant testicular tumor that occurs most frequently in children [77,78,79] and [80]; its myriad synonyms include *endodermal sinus tumor, infantile embryonal carcinoma, orchidoblastoma, Teilum's tumor,* and *clear-cell adenoma*.

From the perspectives of histopathology and natural history, yolk sac tumor of childhood is distinct from its adult counterpart. [77] In children, yolk sac tumor is characteristically pure, whereas adult tumors contain other malignant components. Pediatric testicular yolk sac tumor is localized (stage I) in up to 85% of cases, and the overall survival rate is higher than 70%. [150,265,266] and [267] Weissbach et al.[247] reported survival to be correlated with age (i.e., younger than 2 years vs. 2 years and

older). However, with modern chemotherapy, Mann et al.[150] reported survival of 96% in all prepubertal patients.

Several earlier reports documented increased survival from 60% to 90% in children who had radical orchiectomy and retroperitoneal lymphadenectomy, as compared with those who had orchiectomy alone.[268,269] These series were reported before the improvement of clinical staging with serum markers and CT. Considering that retroperitoneal lymphadenectomy identifies disease in 0% to 20% of cases,[201,252,267] that a significant risk of postoperative ejaculatory dysfunction is present, that serum AFP is elevated in 90% of patients,[265,266] and that two-thirds of patients are cured by orchiectomy alone,[150] the routine use of retroperitoneal lymphadenectomy in stage I patients has been questioned in favor of a more conservative approach.[249,266,269]

After radical orchiectomy, patients are monitored by measurement of serum AFP and periodic evaluation of the chest and abdomen. This process permits early identification of relapses and timely initiation of effective chemotherapy. This conservative approach is being evaluated prospectively in the POG/CCG intergroup study. Even with normalization of AFP, patients with stage I disease must be observed cautiously, because 20% to 40% of false-negative measurements have been identified and retroperitoneal nodal disease has occurred before elevation of AFP.[270,271]

For more advanced disease (stage II or stage III), initial chemotherapy followed by debulking surgery and, possibly, retroperitoneal lymphadenectomy has been suggested in older patients and adults.[272,273] A nerve-sparing modification of retroperitoneal node dissection recently was shown to preserve ejaculation in more than 90% of patients.[269] Because chemotherapy has proven very effective in younger children with this disease, the need for subsequent retroperitoneal lymphadenectomy has been challenged.[249,269,274]

Children with higher-stage disease require postoperative chemotherapy. Regimens containing cisplatin, vinblastine, and bleomycin have improved survival dramatically, even in patients with disseminated disease.[139,140,151,275,276] The addition of ifosfamide and teniposide to these regimens also is promising.[139,277,278] The duration of chemotherapy remains to be determined. Griffin et al.[267] suggested 1 year of treatment, but Einhorn et al.[137] could notdemonstrate the efficacy of extended treatment with vinblastine in advanced disease. Children with residual tumor may benefit from second-look surgery or irradiation.

### Embryonal Carcinoma

The adult-type embryonal carcinoma occurs rarely in young male individuals, usually in late adolescence or early adulthood.[79,80] Reports from the POG[222] and the Pediatric Tumor Registry of Germany[80] documented a 7% incidence among 42 pediatric testicular malignant germ cell tumors. No embryonal carcinomas were noted among 61 cases of testicular cancer in the United Kingdom Children's Cancer Study Group study.[150] The presenting symptoms include an enlarging scrotal mass, metastatic abdominal or mediastinal disease, or localized peripheral lymphadenopathy. Serum AFP or b-HCG (or both) may be elevated, and measurements should be obtained preoperatively.

Initial treatment consists of radical inguinal orchiectomy. On the basis of stage, various combinations of retroperitoneal lymphadenectomy, irradiation, and platinum-containing chemotherapy have been employed. The efficacy of surveillance in stage I tumors and of platinum-containing chemotherapeutic regimens in others is being evaluated in the POG/CCG intergroup trials.

### Teratoma

Teratomas represent 10% of testicular neoplasms in children and occur most frequently before the age of 4 years.[11,102] Approximately15% of these tumors have poorly differentiated elements or immature neuroectodermal components. In prepubescent patients, these features do not impart an adverse prognosis, and essentially all such patients follow a benign clinical course after radical inguinal orchiectomy.[200,201] In contrast, postpubescent testicular teratomas are considered malignant even if these histologic features are not seen. In the postpubertal male person in whom stage II and stage III disease may be observed, the apparently appropriate approach is to perform retroperitoneal lymphadenectomy with nerve sparing and to employ chemotherapy and, possibly, radiotherapy. The use of cisplatin, vinblastine, and bleomycin is associated with a 50% rate of complete response in disseminated disease,[139] with others achieving complete response with chemotherapy plus second-look surgery.

## EXTRAGONADAL TUMORS

Extragonadal germ cell tumors usually occur in midline sites as evidence of *in vivo* alteration in the complex migratory patterns of the embryonal gonads. In order of frequency, the most common locations are sacrococcygeal, mediastinal (including pericardium, heart, and lung), intracranial, retroperitoneal, and uterine. Symptoms relate to the site and histology of the tumor. The POG/CCG intergroup study reported 80% event-free survival and 100% survival with complete resection alone in immature teratomas even when microscopic foci of yolk sac tumor were present.[187] A conservative approach for nonintracranial tumors, similar to that for low-stage gonadal germ cell tumors, also has been reported by the German cooperative study.[279]

### Sacrococcygeal Tumors

Sacrococcygeal teratomas are the most common germ cell tumors of childhood, accounting for 40% of all the germ cell tumors and up to 78% of extragonadal germ cell tumors.[76,78] They also are the most frequently recognized neoplasm of fetuses.[280,281] The histogenesis of these tumors is thought to be related to incomplete migration of germ cells from yolk sac endoderm to the urogenital ridge in presacral tumors or to multipotential embryonic cells associated with the primitive streak (Hensen's node) in postsacral presentations.[282]

Approximately 75% of patients are female.[76,283,284] Congenital anomalies are observed in up to 18% of patients,[76] with musculoskeletal and central nervous system defects being the most common (24% and 26%, respectively).[284] Altmann et al.[76] reviewed the natural history data from 398 cases of sacrococcygeal teratoma and developed a means of classification (Fig. 36-7). Because most of these neoplasms are exophytic and visible to external examination (Fig. 36-8), approximately 80% are diagnosed within the first month of life. These exophytic tumors are less likely to be associated with malignant components.

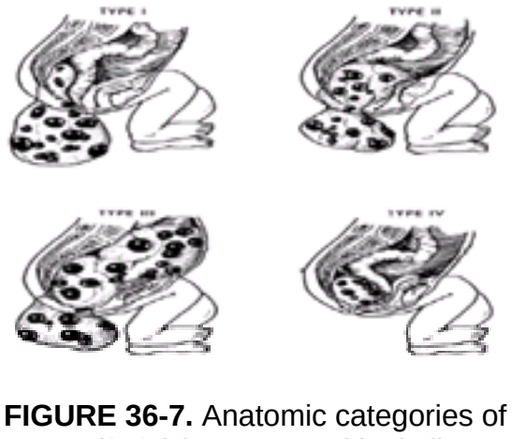


**FIGURE 36-7.** Anatomic categories of sacrococcygeal teratomas. Type I (47%) is a tumor predominantly externalized with limited extension into the sacral region; type II (34%) is a tumor with similar external component and intrapelvic extension; type III (9%) is a tumor with a minimal external component and significant intra-abdominal extension; type IV (10%) is an internalized presacral tumor without external evidence of disease.[76]

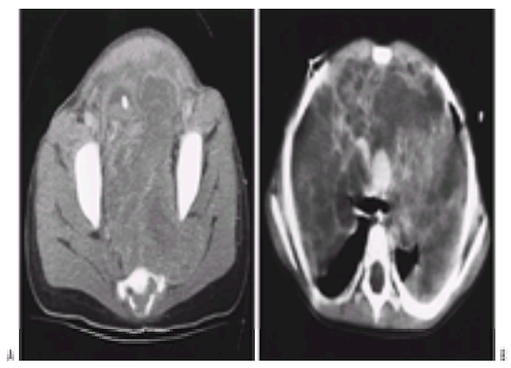

**FIGURE 36-8. A:** Abdominal computed tomographic scan in a 24-month-old girl with a presacral endodermal sinus tumor that fills the pelvis and demonstrates inhomogeneous enhancement after intravenous contrast. Note Foley catheter in compressed bladder and the tumor extending through left sacrosciatic notch. **B:** Chest computed tomographic scan in a 15-month-old girl demonstrating a mediastinal endodermal sinus tumor filling the thorax and showing mixed attenuation at the level of carina.

Approximately 17% of sacrococcygeal teratomas exhibit malignant features.[76,283] The incidence of malignant components is related to surgical type (38% in type IV vs. 8% in type I), age at diagnosis, and gender, but not to the size of the tumor.[76,222] The most common malignant elements identified within the sacrococcygeal lesions are yolk sac tumor and embryonal carcinoma.[75] A histologic grading system similar to that used for immature teratomas has been attempted in extragonadal tumors, but the predictive value of this exercise is not as clear as that in ovarian immature teratoma.[202,204]

Early and complete excision of the presacral germ cell tumor has been the mainstay of successful management.[76] Preoperative establishment of the anatomic boundaries of the tumors, evaluation for evidence of metastatic spread, and assessment of markers (i.e., AFP and b-HCG) for malignancy help to individualize treatment planning. Principles of operative excision that lead to the most successful procedure include (a) sacral incision with removal of the coccyx, (b) early ligation of middle sacral arteries and veins, and (c) circumferential preparation of the torso and lower extremities should intraoperative changes be necessary.[285] The sacrifice of vital organs is not indicated for benign neoplasms, and exenteration of untreated malignant lesions has not proven beneficial.

The cure rate of infants or children with a benign presacral germ cell tumor is more than 95%.[222,285] Cautious monitoring of such patients is required, because malignant germ cell tumors are well recognized to recur either from missed malignant elements in the original tumor or from malignant conversion of residual tissue.[86,282,285,286] Until recently, only a 10% salvage rate for malignant lesions was expected.[274,285,287] With the recognition that excision of primary malignant lesions alone has cured few patients, the addition of chemotherapy, particularly platinum-containing regimens addressing the specific malignant element, has improved survival.[168,222,288,289 and 290]

### Mediastinal Tumors

Thoracic germ cell tumors usually (although not exclusively) are located in the anterior mediastinum.[291,292,293,294 and 295] They are more common in male individuals.[296,297,298,299 and 300] Adolescents typically present relatively asymptomatically, in contrast to infants and toddlers, who more often exhibit severe respiratory symptoms, including hemoptysis.[301,302 and 303] Teratoma subtypes (mature, immature, or malignant) comprise the bulk of the histologic subtypes seen, but yolk sac tumor and choriocarcinoma occasionally may be detected.[293,294 and 295,297,301] Mediastinal teratomas occasionally have sarcomatous foci resembling rhabdomyosarcoma, angiosarcoma, or undifferentiated sarcoma.[81] These foci are extremely aggressive, tend to overgrow the remaining teratoma, and create difficulty in treatment. Therefore, appropriate metastatic surveys for these tumors and assessment of serum markers (AFP, b-HCG, LDH, and PLAP) are recommended. Mediastinal germ cell tumors frequently are associated with Klinefelter's syndrome (47,XXY).[304]

Malignant germ cell tumors, usually with a yolk sac tumor component, are associated also with hematopoietic malignancies.[305,306 and 307] The source of these malignancies is controversial, but studies have shown that the cells in the hematopoietic malignancy have cytogenetic and molecular genetic identity and are immunohistochemically similar to pluripotential cells within the germ cell tumor rather than the host's own bone marrow cells. This evidence supports the contention that the germ cell tumor cells—and not the patient's host marrow—are the source of the second malignancy.[67,108,305,308,309] Very rarely, such malignancies have been associated with gonadal tumors as well[75]; in most such cases, affected patients have been shown to have some form of XY gonadal dysgenesis. More recent pediatric studies reporting outcome specifically for mediastinal subsets of children and adolescents with malignant germ cell tumors treated with platinum-based regimens suggested that although this site is considered less favorable, event-free survival of 57% to 88% was achieved.[155,156 and 157]

### Intracranial Tumors

Primary intracranial germ cell tumors may be located in the pineal gland (62%) or suprasellar region (31%), or they may span both areas (7%).[310,311] Symptomatology depends on the growth pattern and histology of the tumor and may include visual disturbances, diabetes insipidus, hypopituitarism, Parinaud's syndrome (convergence nystagmus), anorexia, and precocious puberty.[312,313] Histologically, two-thirds of the tumors are germinomas, and the rest are nongerminomatous, some mixed with yolk sac tumor, choriocarcinoma, or teratocarcinoma.[311,313] Drop metastases to the spine and extracranial spread to lung and bone have been reported.[310,314] Serum levels of AFP and b-HCG should be measured, because they are elevated in some patients.[311]

Initial management should include attempts to obtain a biopsy and to resect the primary tumor so that appropriate subsequent therapy may be planned. Germinomas traditionally have been treated with radiotherapy. More recently, studies by Finlay et al.[315] reported that many of these tumors can be managed with a carboplatin-based chemotherapeutic regimen.

### Tumors at Other Sites

Other extragonadal sites of origin of germ cell tumors include retroperitoneum, pelvis, cervix or uterus, vagina, prostate, abdominal wall, bile duct, omentum, hernia sac, and lip.[150,152,222,316,317,318,319 and 320] Vaginal lesions must be differentiated from vaginal rhabdomyosarcoma. Although metastatic bone disease is infrequent, it is reported in 3% of adult patients with newly diagnosed disease and in 9% of those with relapses.[321]

## FUTURE CONSIDERATIONS

During the last decade, the progress in understanding and managing germ cell tumors of children has been substantial. The intergroup (POG/CCG) studies in the United States and the evolving trials in Europe and the United Kingdom demonstrate clearly that the next challenge will be to refine further our comprehension of these diverse tumors through the development of risk groupings. As with acute lymphocytic leukemia and neuroblastoma, the continued focus on the molecular and genetic facets of pediatric germ cell tumors promises to yield data that will explain and predict the diverse behavior of these tumors and perhaps lead to the development of unique treatment strategies. Further trials are being proposed to investigate the role of chemoprotective agents, such as amifostine, to reduce ototoxicity in young children receiving higher doses of cisplatin. The development of less toxic chemotherapeutic combinations is a high priority for all patients but certainly for lower-risk patients who require some postoperative treatment. For high-risk disease, dose-intensive therapy with autologous marrow re-infusion requires further assessment. Newer agents, such as ifosfamide, carboplatin, and perhaps topotecan, will be investigated further in patients with relapses and should furnish the basis of future combination regimens.

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# 37

# ENDOCRINE TUMORS

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## INTRODUCTION

Endocrine tumors are those neoplasms arising from endocrine organs, regardless of whether they secrete hormones, and those tumors secreting hormones, regardless of the tissue of origin. The secretion of hormones by neoplasms of nonendocrine origin or by neoplasms arising from glands that normally secrete different hormones is called *ectopic production*.[1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20 and 21] Endocrine tumors represent approximately 4% to 5% of all neoplasms observed in children.[22,23,24 and 25] Most of these tumors do not secrete hormones.

Approximately 40% to 45% of childhood endocrine tumors arise from the gonads (e.g., germ cell, testicular, and ovarian tumors), 30% arise from the thyroid gland, and 20% arise from the pituitary gland. The others arise from the parathyroid glands, the adrenal cortex and medulla, the gastroenteropancreatic system, and nonendocrine tissues, such as the thymus.

Most endocrine tumors in childhood are clinically benign or low-grade malignancies. A small percentage of gonadal and germ cell tumors, thyroid neoplasms, and adrenocortical tumors are high-grade malignancies. Malignant carcinomas of the parathyroids, the adrenal medulla, and the gastroenteropancreatic system are rare.

Some endocrine tumors in childhood are of embryonic origin (e.g., germ cell tumors, craniopharyngiomas). Although most neoplasms that occur in children are sporadic, a small portion of certain endocrine tumors are familial, with some transmitted in a mendelian fashion. For instance, medullary carcinomas of the thyroid gland and pheochromocytomas can occur in a familial syndrome called *multiple endocrine neoplasia type II* (MEN II). MEN II is transmitted in an autosomal dominant fashion with high penetrance.[26] MEN type I (MEN I) is also a familial endocrine cancer syndrome. Patients with MEN I commonly have tumors of the parathyroid glands and pituitary gland.[4,5 and 6,27] The association of skin or heart myxomas, spotty skin pigmentations (e.g., lentigines), and multiple endocrine tumors is known as *Carney complex*, a condition that is inherited in an autosomal dominant manner.[28] Other genetic disorders, such as neurofibromatosis type 1 (NF-1) and von Hippel-Lindau (VHL) disease, frequently are associated with endocrine tumors, most commonly pheochromocytoma.[29]

This chapter discusses the endocrine tumors of the hypothalamic-pituitary unit, the thyroid gland, the parathyroid glands, the adrenal cortex and medulla, the adrenergic ganglia, and the gastroenteropancreatic system (e.g., insulinomas, gastrinomas, glucagonomas, VIPomas, and somatostatinomas). Also reviewed are ectopic hormone–secreting tumors and the MEN syndromes. Several features of these tumors are summarized in Table 37-1. Gonadal and germ cell tumors and carcinoid tumors are considered in Chapter 36 and Chapter 38.

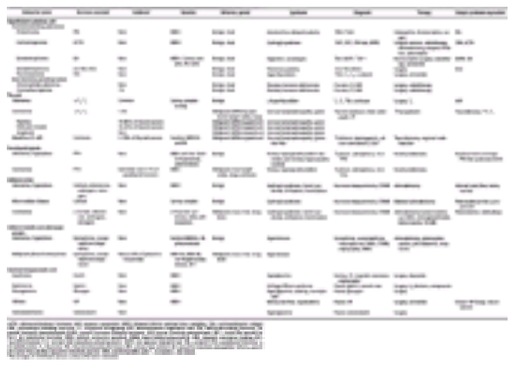


**TABLE 37-1. ENDOCRINE TUMORS IN CHILDREN AND ADOLESCENTS**

## TUMORS OF THE HYPOTHALAMIC-PITUITARY UNIT

Most tumors occupying the pituitary fossa are benign. The two main types include craniopharyngiomas and pituitary adenomas. In childhood, tumors that metastasize to the pituitary gland are exceedingly rare. Pituitary functions may be affected by neoplastic or infiltrative processes of the pituitary area, including gliomas arising from the optic chiasm or surrounding region; germ cell tumors arising from the pituitary stalk; meningiomas; and granulomatous diseases that include histiocytosis X, tuberculosis, and sarcoidosis.

The most common tumor of the pituitary fossa is the craniopharyngioma, which is a nonsecretory tumor that represents approximately 5% of all intracranial tumors in childhood and accounts for approximately 90% of neoplasms arising in the pituitary region. In contrast, pituitary adenomas comprise approximately 3% of supratentorial tumors in childhood or 6% of all surgically treated pediatric pituitary tumors. The remaining tumors are rare ( Table 37-1).[2,3,30,31,32,33,34,35 and 36] Hypothalamic hormone-secreting tumors are infrequent and usually affect adults. [37,38 and 39] However, hypothalamic hamartomas and hamartoblastomas occur in children. These tumors are nonsecretory but cause endocrine symptoms (e.g., precocious puberty) by disrupting normal hypothalamic function. [40,41 and 42]

Pituitary tumors that secrete hormones take the name of the hormone they secrete and the ending -*oma*. Prolactinomas, corticotropinomas, somatotropinomas, gonadotropinomas, and thyrotropinomas secrete, respectively, prolactin, adrenocorticotropic hormone (ACTH), somatotropin [i.e., growth hormone (GH)], gonadotropins [i.e., luteinizing hormone (LH), follicle-stimulating hormone (FSH)], and thyroid-stimulating hormone [thyrotropin (TSH)]. Each of these tumors usually produces a characteristic syndrome because of excess hormone secretion.

In the past, pituitary adenomas that did not secrete hormones and that did not stain by acid or basic stains were called *chromophobe adenomas*. Currently, immunohistochemistry is used to classify pituitary adenomas: For instance, in a patient with clinical and biochemical features of ACTH-dependent Cushing's syndrome, pituitary tissue that stains positively for ACTH indicates an ACTH-secreting pituitary adenoma. Chromophobe adenomas now are called *nonsecreting, hormonally inactive pituitary adenomas*, which account for 6% of pituitary adenomas that occur in children, as opposed to adults, in whom these adenomas account for approximately one-third of all pituitary adenomas. Fewer than 25% of pediatric patients with pituitary adenomas present before age 12. [43,44 and 45]

At present, the imaging modality of choice for detecting pituitary adenomas is the T1-weighted spin-echo magnetic resonance imaging (MRI) of the pituitary before and after administration of gadolinium. Before and after contrast, coronal and sagittal images should be obtained at 3-mm intervals focusing on the pituitary region. Pituitary adenomas usually appear as hypoenhancing lesions, as they are slowly taking up gadolinium compared to the surrounding normal pituitary tissue. In interpreting pituitary MRI scans in children, one should take into account that the normal pituitary gland may be enlarged normally during adolescence. Microadenomas, defined as pituitary adenomas smaller than 1 cm, are detected by the presently available MRI technology in only approximately 50% of cases. The detection rate of pituitary adenomas has increased over the last three decades as a result of improved imaging modalities. In a retrospective survey over 33 years, the median survival of patients with pituitary adenoma was 18 years for men and 25 years for women. Most common causes of death in patients with pituitary adenomas were cardiovascular or cerebrovascular.[46]

### Clinical Presentation

All pituitary tumors may cause symptoms arising from pressure on the adjacent structures. Headaches, visual disturbances, and manifestations from one or more hypothalamic-pituitary hormone deficiencies can be the presenting symptoms. Hyperprolactinemia may develop as a result of deficiency of the hypothalamic tuberoinfundibular dopaminergic system, which is responsible for suppression of prolactin secretion. In adolescent girls with hyperprolactinemia, menstrual dysfunction and galactorrhea may occur. Intracranial hypertension and hydrocephalus may develop in patients with (large) pituitary tumors. The usual finding on ophthalmologic examination is bitemporal constriction of the visual fields ( Fig. 37-1), because most suprasellar pituitary tumors impinge on the crossing fibers of the optic chiasm. The optic nerve fibers are arranged according to size, with the larger occupying a more inferior location, whereas smaller macular fibers occupy a more superior location. An ipsilateral central scotoma with a normal contralateral visual field is the most consistent finding on visual field testing. Other changes in the visual fields depend on the location of the tumor. Compression of the optic chiasm can produce a characteristic ophthalmoscopic optic nerve appearance of band or “bow-tie” atrophy.

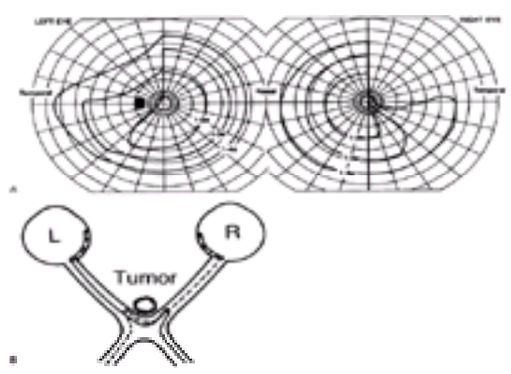



**FIGURE 37-1.** Visual field defects commonly observed with pituitary tumors. Impinging of the tumor on the anterior optic chiasm **(A)** results in bilateral superior temporal quadrantopia **(B)** owing to involvement of the lower anterior crossing nasal fibers. Further involvement of the anterior optic chiasm results in bilateral temporal hemianopia. Lateral or posterior location of the tumor results in contralateral nasal or bilateral inferior temporal defects of the visual fields, respectively.

Deficiency of one or more pituitary or hypothalamic hormones may result from pressure or encroachment by the pituitary tumor. Depending on the hormone affected, different symptoms may arise. The tests required for the diagnosis of pituitary deficiencies are summarized in Table 37-2.[7,9,14,33,47] The hormone affected most frequently is GH. Deficiency of this hormone leads to poor growth, hypoglycemia, or both in younger children. The deficiency is diagnosed by measuring the plasma GH concentration after stimulation with arginine-insulin, L-dopa, or glucagon. The attainment of symptomatic hypoglycemia (obtained by administration of 0.1 units per kg of regular insulin as an intravenous bolus) is a prerequisite for evaluating lack of response. Two abnormal test results (i.e., plasma GH elevations less than 6 ng per mL) are required for the diagnosis. Priming with gonadal steroids is recommended before GH testing in prepubertal children.

| Hormone | Tests | Response |
|---|---|---|
| GH | Arginine or insulin stimulation | GH ≥6 ng/mL |
| | L-Dopa stimulation | GH ≥6 ng/mL |
| | Glucagon stimulation | GH ≥6 ng/mL |
| ACTH | Cortrosyn stimulation | Cortisol ≥18 µg/dL |
| LH, FSH | LH-releasing hormone stimulation | LH and FSH normal for age |
| TSH | Plasma $T_4$, $FT_4$, $T_3$, TSH, TBG | |
| | TRH stimulation | TSH normal for age |
| AVP | Water deprivation | Urinary osmolality ↓<br>Serum osmolality ↑ |

ACTH, adrenocorticotropic hormone; AVP, aqueous vasopressin; FSH, follicle-stimulating hormone; $FT_4$, free thyroxine; GH, growth hormone (somatotropin); LH, luteinizing hormone; $T_3$, tri-iodothyronine; $T_4$, thyroxine; TBG, thyroxine-binding globulin; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; ↓, decreased; ↑, increased.

**TABLE 37-2. ENDOCRINE TESTS FOR EVALUATION OF PITUITARY DEFICIENCIES**

Deficiency of ACTH leads to secondary adrenal insufficiency characterized by weakness, orthostatic hypotension, hyponatremia (if chronic lack of ACTH with subsequent atrophy of the adrenal cortex), and hypoglycemia in younger children. It is a life-threatening condition that must be diagnosed early. Diagnosis is made by measuring plasma cortisol 1 hour after adrenal stimulation with an intravenous bolus of 10 µg per kg of ACTHs 1–24 [cosyntropin (Cortrosyn)]. A normal response (i.e., plasma cortisol greater than 18 µg per dL) indicates normal function of the adrenal glands. Long-term pituitary ACTH deficiency produces adrenal atrophy and a diminished cortisol response.

Gonadotropin and TSH deficiencies may occur in patients with pituitary tumors. Gonadotropin deficiency manifests as pubertal arrest or regression in children already in puberty or manifests as delayed puberty in children affected prepubertally. Plasma LH and FSH are low for the age in these children, and bone age is delayed. TSH deficiency manifests as poor growth, diminished performance at school, constipation, cold intolerance, dry skin, and other symptoms of hypothyroidism. Measurements of serum total thyroxine ($T_4$), free $T_4$, and TSH are required for the diagnosis.

Extension of a pituitary tumor into the hypothalamic paraventricular nucleus may result in aqueous vasopressin (AVP) deficiency and diabetes insipidus (e.g., polyuria, polydipsia, dehydration, hypernatremia). The diagnosis of diabetes insipidus may be confirmed by a water deprivation test.

Certain hypothalamic tumors (e.g., hamartomas) can present with precocious puberty or hyperprolactinemia caused by compression of the pituitary stalk with subsequent increased prolactin secretion and by disruption of the normal inhibition of puberty. Hamartomas are common in genetic syndromes associated with craniofacial anomalies and abnormalities of the extremities. Patients with these genetic defects or a positive family history should be screened for these tumors by MRI.[40,41]

### Endocrine Manifestations of Hormone-Secreting Tumors

#### *Prolactinomas*

The type of pituitary adenoma that occurs most frequently in children is the prolactinoma, accounting for 53% of all tumors. Prolactinomas commonly stain for acidophilic cells and often, in addition, for GH by immunohistochemistry. The majority of such tumors in children is detected during the adolescent period. Most prolactinomas are microadenomas. Age and gender of the child determine the type of tumor presentation. Prepubertal children typically suffer from headaches, visual disturbances, or growth failure. Pubertal girls commonly show symptoms of pubertal arrest or delay, hypogonadism, or galactorrhea caused by suppression of gonadotropin secretion. Galactorrhea may not occur spontaneously and must be excluded by expressing the breast. Pubertal boys also may present with pubertal arrest or delay or growth failure (or both). Gynecomastia is not frequent. Prolactinoma should be separated from secondary hyperprolactinemia, which may be caused by any disorder that leads mechanically (e.g., hypothalamic tumors), neurogenically (e.g., nipple stimulation), or pharmacologically (e.g., antidopaminergic drugs) to loss of dopaminergic suppression of the pituitary lactotropes. Because prolactinomas have a diverse clinical picture, prolactin levels should be measured according to a low threshold. The upper limit of normal for most laboratories is 30 ng per mL. Random prolactin levels that are elevated to less than 50 ng per mL should be repeated (fasting levels, an hour after placement of an indwelling cannula). If the prolactin elevation persists, reversible factors that may cause hyperprolactinemia should be investigated further and excluded before the start of treatment (Table 37-3).[6,32,34,48,49] Prolactinomas may be seen in children with the McCune-Albright syndrome (i.e., precocious puberty, cutaneous café-au-lait spots, and polyostotic fibrous dysplasia) and in young adults with MEN I.

**TABLE 37-3. ENDOCRINE TESTS FOR EVALUATION OF HORMONE EXCESS IN CHILDREN AND ADOLESCENTS**

Medical therapy with dopamine agonists is the first line of treatment before surgical therapy, in the absence of such surgical emergency as an acute threat to vision, hydrocephalus, or cerebrospinal fluid leak. Bromocriptine is especially effective in treating smaller tumors. Its main side effects are gastrointestinal (GI) disturbances and postural hypotension. At least as efficacious as bromocriptine at reducing tumor size and prolactin levels is pergolide; it, however, has a side effect profile similar to that of bromocriptine. A newer ergot-dopamine agonist—cabergoline—causes fewer side effects than do bromocriptine and pergolide and is to be administered once or twice weekly. Rarely, prolactinomas are medically refractory. In these situations, dopamine receptor imaging can be used to gain insight into the mechanisms of drug failure.[34,50,51,52 and 53]

#### *Corticotropinomas*

Corticotropinomas are the second most common pituitary adenomas in children and the most common tumor seen prepubertally.[44,45] Most corticotropinomas are microadenomas, although diffuse corticotropic cell hyperplasia also has been described. Excess pituitary ACTH secretion (i.e., Cushing's disease) produces hypercortisolism and the characteristic features of Cushing's syndrome, including growth arrest, pubertal arrest, weight gain, hypertension, the characteristic phenotype (i.e., moon facies, buffalo hump, accumulation of supraclavicular fat), acne, purple skin striae, and weakness. Skin thinning and easy bruising are uncommon, in contrast to effects seen in adult patients with hypercortisolism. Many adolescent and young adult patients complain of mood disturbances.[54,55] However, instead of experiencing depression, memory problems, and sleep disturbances, children with Cushing's syndrome often tend to be obsessive and are high performers in school.

Corticotropinomas account for 85% of children who are older than 5 years and develop Cushing's syndrome. Other causes for hypercortisolism are primary adrenal tumors, ectopic ACTH production (bronchial or thymic carcinoids), and ectopic or eutopic corticotropin-releasing hormone–producing tumors. In children and adolescents, pseudo-Cushing's syndrome is extraordinarily rare.

The diagnosis of hypercortisolism is made by measuring 24-hour urinary free cortisol (UFC) and 17-hydroxysteroid excretion in conjunction with urinary and plasma creatinine concentrations. UFC values should be corrected for children's body surface area (usually per 1.73 $m^2$). Cushing's syndrome is confirmed further by a midnight low-dose dexamethasone (15 µg per kg) suppression test with failure of serum cortisol to suppress to less than 3 µg per dL at 8 a.m. The diagnosis of pituitary Cushing's disease is made by the following series of endocrine tests ( Table 37-3).

**Corticotropin-Releasing Hormone Test**

Measurements of plasma ACTH and cortisol are obtained before and after intravenous administration of ovine corticotropin-releasing hormone (oCRH; 1 µg per kg).[55,56 and 57] Most patients with pituitary Cushing's syndrome respond with elevations of ACTH and cortisol, but most patients with the other categories of Cushing's syndrome do not respond.

**Liddle Dexamethasone Suppression Test**

In the Liddle dexamethasone suppression test,[54,55] patients undergo six sequential 24-hour urine collections for measurement of 17-hydroxysteroids or UFC. The first two collections provide the baseline. During the next 2 days, affected patients are given 2 mg dexamethasone per day orally (30 µg per kg per day) in divided doses every 6 hours. During the last 2 days, such patients are given 8 mg dexamethasone per day orally (120 µg per kg per day) in divided doses every 6 hours.

Patients with Cushing's disease show characteristic suppression of their 17-hydroxysteroid or UFC excretion to less than 50% and 90% of baseline levels, respectively, only on the high dose of dexamethasone. At this dose, neither patients with ectopic ACTH secretion nor patients with cortisol-secreting adrenal adenomas or carcinomas respond with suppression. A suppression of serum cortisol by more than 50% after the administration of 120 µg per kg of dexamethasone (high dose) given at midnight has a sensitivity of 80% for the diagnosis of Cushing's disease. This sensitivity is comparable with that (85%) of the formal 6-day Liddle test. The advantages of the “short” high-dose dexamethasone suppression test are low costs and patient convenience, as the test does not require patient hospitalization or timed urine collections.

**Bilateral Inferior Petrosal Sinus Sampling**

If the pituitary MRI result is negative and other tests indicate ACTH dependence, oCRH-stimulated bilateral inferior petrosal sinus sampling can be used to confirm that the source of ACTH secretion is the pituitary gland.[56,57 and 58] In addition, this procedure can assist in lateralizing the adenoma with 75% accuracy. The two inferior petrosal sinuses, which drain the pituitary gland, are catheterized, and blood for measurements of ACTH is obtained simultaneously from both and from a peripheral vein. The presence of a concentration difference between either of the petrosal sinuses and the peripheral vein (ratio greater than 1.6) confirms the pituitary source of ACTH. A difference in concentration between the two petrosal sinuses (ratio greater than 1.6) suggests the location of the adenoma. The sensitivity of this test at confirming pituitary ACTH dependence is 97%.

To improve or simplify, respectively, the oCRH-stimulated inferior petrosal sinus sampling, sampling of both cavernous sinuses or the internal jugular veins has been investigated, with inconclusive results thus far.[59,60,61,62 and 63] The treatment of choice for corticotropinomas is surgical excision. For patients with noninvasive microadenomas, the cure rate is 90%, and the recurrence rate is less than 10%. For patients with large or invading tumors (e.g., cavernous sinuses), the chance of cure is still very high, but recurrence rates are greater than 10%. After removal of the adenoma, affected children become hypocortisolemic and require replacement therapy with hydrocortisone (which entails 8 to 10 mg per $m^2$ per day given in the morning, education about stress dosing of hydrocortisone, and an emergency kit of intramuscular hydrocortisone) for 6 to 12 months until the remaining and transiently suppressed nontumorous pituicytes resume appropriate ACTH secretion. Normal pituicyte function for ACTH can be confirmed by a cortisol response of more than 18 µg per dL, 30 minutes after stimulation with 250 µg cosyntropin. In patients with unresectable adenomas or more than two recurrences, radiotherapy can produce normalization of cortisol, often in association with delayed plurihormonal pituitary hormone deficiency. Approximately 80% of affected children treated with fractionated radiation doses of 35 to 50 cGy obtain cure over a 2-year interval.[34,64,65]

The molecular genetics of corticotropinomas and pituitary adenomas in general is the subject of intense investigation. Apparently, pituitary adenomas (including corticotropinomas) are monoclonal in origin. Studies of the involvement of proto-oncogenes in tumorigenesis of corticotropinomas have been disappointing, although some genes, such as *ras*, c-*erb* B-2, c-*myc*, and protein kinase C have been associated with more aggressive tumors. Abnormalities of the p53 tumor suppressor gene were found in as many as 60% of corticotropinomas but, to date, the role of this tumor suppressor gene in tumorigenesis of corticotropinomas remains unclear, as does the role of other tumor suppressor genes, including *p16, CDKN2*, *MTS1*, *INK4*, *RB1*, *p27*, *KIP1*, *CDKN4*, *hZAC*, and *NM23*. In patients with MEN I, corticotropinomas occasionally occur, although the typical pituitary adenoma in this familial tumor syndrome is a prolactinoma. For corticotropinomas and for other pituitary adenomas, the exact pathway of tumor formation remains unknown, as does the role of tumor-promoting or acquired factors.[66,67,68,69,70 and 71]

***Somatotropinomas***

GH-secreting adenomas account for some 15% of pediatric pituitary tumors. Most somatotropinomas in children and adults are diagnosed as macroadenomas. In addition, GH excess can be caused by somatotrope hyperplasia. Such hyperplasia can occur in combination with hypothalamic or ectopic tumors that secrete GH-releasing hormone. Gigantism or acromegaly has been observed in children with the McCune-Albright syndrome and in young adults with MEN I and Carney complex. Activating mutations of the Gsa subunit of the guanine nucleotide-binding proteins (G proteins) were reported for approximately one-half of sporadic somatotropinomas.[73,74]

GH excess before epiphyseal fusion leads to accelerated growth and gigantism and, after closure of the epiphyses, to acromegaly. In addition to the development of the characteristic acromegalic phenotypes, hypersecretion of GH can be associated with carbohydrate intolerance or frank diabetes, arthropathy, and the carpal tunnel syndrome.[72] In children with GH excess, an increase in colonic polyposis, malignancy, or thyroid nodules has not yet been reported, as it has in adults. In addition to the foregoing symptoms, some somatotropinomas may cause severe headaches and visual disturbances. The diagnosis is made by measuring insulin-like growth factor-1 (IGF-1) levels, which will be elevated in patients with GH excess. However, some overlap is seen with IGF-1 levels during normal puberty and pregnancy. Measuring a random GH level may be helpful, as is the determination of plasma levels of GH before and after oral ingestion of glucose (1.75 g per kg) in a glucose tolerance test. In patients with GH excess, the plasma GH concentration does not decrease after glucose administration and, paradoxically, may even rise, in contrast to the result in normal individuals, who will develop low to nonmeasurable GH concentrations after glucose administration ( Table 37-3). Somatotropinomas usually are large pituitary adenomas that are easily detected on MRI. Virtually all GH-producing pituitary adenomas possess somatostatin receptors, thus allowing for somatostatin receptor imaging with the analog octreotide. However, other pituitary tumors and pituitary metastases from somatostatin receptor–positive neoplasms, parasellar meningiomas, lymphomas, or granulomatous diseases of the pituitary also may be positive on somatostatin receptor imaging with octreotide. Furthermore, an octreoscan does not seem to have a role in deciding whether to treat pediatric patients with octreotide.[75,76] The role for an octreoscan lies more in detecting ectopic tumors that secrete pituitary hormones or hypothalamic-releasing hormones, such as GH-releasing hormone. Traditionally, surgery has been the treatment of choice for patients with GH excess from a somatotropinoma, with irradiation reserved for patients who were not surgically curable. In adults with acromegaly, long-term trials of octreotide resulted in suppression of GH to less than 5 µg per L in 65% and to less than 2 µg per L in 40%, with normalization of IGF-1 levels in 56% of cases. A drawback of octreotide therapy is the need for several daily injections. The dose required to suppress GH levels varies, depending on tumor size and receptor expression on tumor cells.

Alternatively, a long-acting, slow-release depot somatostatin analog—lanreotide—has come into use and can be administered once every 2 weeks. This drug is as effective as octreotide and successfully suppresses GH and IGF-1 levels. Medical therapy with dopamine agonists, such as bromocriptine, has been disappointing, with normalization of IGF-1 levels in only approximately 10% of patients affected by GH excess. Recently, treatment with the GH receptor antagonist pegvisomant has been shown to be potentially useful for patients with acromegaly.[77,78,79,80 and 81]

***Gonadotropinomas***

Hypersecretion of LH in prepubertal boys could lead to precocious puberty because of the excess stimulation of the Leydig cells and excessive production of testosterone. Tumors of this type, however, have yet to be described in children. Intracranial tumors that produce human chorionic gonadotropin, which has LH activity, include such germ cell tumors as choriocarcinomas, mixed germ cell tumors, germinomas, and embryonal carcinomas. These tumors lead to precocious puberty only in boys and generally are small and detectable mostly by MRI. Human chorionic gonadotropin produced by these tumors can be detected in the

cerebrospinal fluid. Gonadotropinomas secreting FSH are associated with visual disturbances and hypogonadism. [82,83 and 84]

### *Thyrotropinomas*

TSH-secreting pituitary adenomas are rare in adults and even rarer in children. Because of delayed diagnosis, these tumors often have been detected as macroadenomas leading to headaches and visual disturbances in affected patients. Newer imaging modalities (MRI) and improved biochemical hormone assays allow earlier diagnosis of these tumors. Hypersecretion of TSH can lead to hyperthyroidism. Plasma TSH, total $T_4$, free tri-iodothyronine ($T_3$), and free $T_4$ levels are elevated (Table 37-3).[85,86] This acquired syndrome should be differentiated from the genetic syndrome of pituitary resistance to thyroid hormone, with which it is biochemically identical.[85,86] Unlike patients with $T_4$ resistance, patients with thyrotropinomas usually do not respond to TSH-releasing hormone stimulation. This test has a sensitivity of 71% and a specificity of 96%. In distinction to patients with pituitary thyroid hormone resistance, patients with TSHomas have an elevated a-glycoprotein subunit (specificity, 90%; sensitivity, 75%) and an elevated ratio of alpha subunit to TSH (83% sensitivity, 63% specificity). [86,87] Treatment of choice for TSHomas is transsphenoidal surgery, often in conjunction with radiotherapy when such tumors prove large and invasive. Octreotide administration can normalize thyroid hormone levels in 80% of patients and can lead to tumor shrinkage in approximately 50% of cases, especially for residual or recurrent tumor. [86,87,88 and 89]

## Non–Hormone-Secreting Tumors

### *Chromophobe Adenomas*

Hormonally inactive pituitary adenomas are rare tumors that account for only 6% of pediatric pituitary adenomas, as opposed to 30% in some adult series. These tumors usually are discovered incidentally when affected patients undergo brain imaging for other reasons or present for evaluation of hyperprolactinemia, headaches, growth or pubertal failure, or visual disturbances. At diagnosis, these tumors usually are large and may cause elevations of plasma prolactin concentrations as a result of pituitary stalk compression and insufficient dopaminergic suppression of the lactotrophs. [90] These tumors occasionally secrete the alpha subunits of glycoprotein hormones (e.g., TSH, LH, FSH), which are hormonally inactive. They also may secrete the b-glycoprotein hormone subunits and chromogranin A.[34,91] The mainstay of therapy is surgery, if symptoms are present or arise. Because distinguishing these tumors from craniopharyngiomas is difficult, the threshold for surgical intervention in children likely is low.

### *Craniopharyngiomas*

Craniopharyngiomas account for 90% of neoplasms in the pituitary region and arise from remnants of Rathke's pouch, the anlagen of the adenohypophysis. These tumors may develop at any time in life but have a bimodal peak in incidence: one at 5 to 14 years of age and another after age 50. [34,35] Two-thirds of the tumors are calcified and can be seen on plain radiographs. Cystic components are common. Craniopharyngiomas expand locally, and symptoms reflect their location and pressure on or destruction of local structures. Headaches, visual disturbances, and panhypopituitarism with diabetes insipidus are frequent manifestations. Most headaches likely develop from stretching of the diaphragma sellae by the enlarging mass. Up to 75% of patients have GH deficiency, followed by gonadotropin deficiency in 40% and ACTH and TSH deficiency in 25%.

Despite the often large size of the tumor, the pituitary stalk is disrupted uncommonly, with approximately 20% of patients suffering from mild hyperprolactinemia due to the inability of hypothalamic (tuberoinfundibular) dopamine to reach the lactotrophs and act as a prolactin-inhibiting factor. [92] Also uncommon is the loss of the posterior pituitary function, with perhaps 15% of patients presenting with diabetes insipidus. [93,94]

The treatment of choice is surgery. Surgical management depends on many factors, including a patient's age, type of presentation (e.g., acute, hydrocephalus, existing hypothalamic problems), and tumor size. Some patients may require external fractionated radiotherapy with doses up to 56 Gy. Postoperatively, endocrine dysfunction frequently occurs regardless of the surgical approach. In predominantly cystic tumors, adjunctive localized intracavity yttrium, $^{32}P$, and other radioactive implants have proven useful for recurrent tumors. [95,96,97,98,99 and 100]

### *Hypothalamic Hamartomas*

Hypothalamic hamartomas are congenital malformations consisting of ectopic neurons of variable maturity and glial cells irregularly arranged in fibrillary matrix. They arise from cells forming the hypothalamic sulcus that divides the alar plate facing the lumen of the diencephalon into a dorsal (thalamus) and ventral (hypothalamus) region. Hypothalamic hamartomas constitute the most common identifiable central nervous system lesion causing central precocious puberty. Sometimes these tumors are associated with fits of laughter and often manifest as pedunculated masses at the base of the hypothalamus on MRI.

In one case of hamartoma, a significant amount of gonadotropin-releasing hormone was found in the tumor. [101] Most hamartomas, however, lead to precocious puberty by abolishing the normal hypothalamic control of the onset of puberty.

Hypothalamic hamartomas frequently are seen in the context of genetic syndromes associated with craniofacial and skeletal anomalies. The Pallister-Hall syndrome is autosomal dominant and is characterized by orofacial, cardiac, lung, renal, genital, anal, and limb malformations, such as postaxial polydactyly. Reduced penetrance has been found in some families with this syndrome. Screening for hypothalamic hamartoma by MRI is recommended. Other genetic conditions associated with hypothalamic hamartomas include the oral-facial-digital syndrome type VI (Varadi's syndrome) and several cases of holoprosencephaly. [40,41,102]

## Treatment and Prognosis

Surgery always is recommended for pituitary tumors that enlarge rapidly and threaten vision, regardless of the type of the tumor. Smaller tumors can be removed by transsphenoidal surgery. Larger tumors with suprasellar extensions can be removed by craniotomy or by combined craniotomy and transsphenoidal surgery. Frequently, complete tumor excision is not feasible, and mere debulking is attempted.

Transsphenoidal surgery is a low-risk procedure (Fig. 37-2).[101,102,103,104,105,106,107 and 108] Rare complications include total hypophysectomy and panhypopituitarism; cavernous sinus hemorrhage; transient or permanent diabetes insipidus or inappropriate antidiuretic hormone secretion syndrome; cerebrospinal fluid leaks; and meningitis.

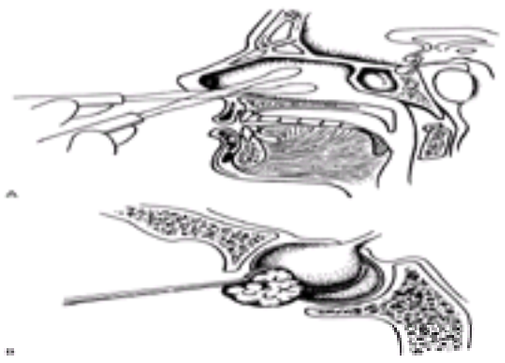


**FIGURE 37-2.** Transsphenoidal surgery. The pituitary gland is approached through the sphenoid sinus **(A)**. Transsphenoidal exposure of the pituitary gland and adenomectomy **(B)**. (Adapted from Hardy J. Transsphenoidal surgery of hypersecreting pituitary tumors. In: Kohler PO, Ross GT, eds. Diagnosis and treatment of pituitary tumors. New York: American Elsevier, 1973:179.)

Transsphenoidal adenomectomy is the treatment of choice for corticotropinomas.[109,110 and 111] Lateralization of the plasma ACTH concentration difference during bilateral sampling of the inferior petrosal sinuses and MRI of the sella turcica usually help with localizing the adenoma to one side of the pituitary. [57,58] In addition, selective venous sampling from the cavernous sinuses and intraoperative ultrasonography may help. [60,61,108] Some corticotropinomas cannot be differentiated from normal pituitary tissue during surgery, for which hemipituitectomy-hemihypophysectomy of the side of the higher ACTH concentration is recommended.

In the largest reported series of pediatric patients with corticotropinomas, remission of hypercortisolism was achieved in 48 of 49 who underwent transsphenoidal surgery.[109] Nineteen percent of these patients had one or more endocrine deficiencies postoperatively. Central hypothyroidism and diabetes insipidus were transient, both resolving within the first 2 years postoperatively in most patients. Suppression of spontaneous GH secretion occurred in all children and adolescents with Cushing's disease during their illness and up to 1 year after surgical cure, contributing to the reduced final adult height of these individuals. [110,112] These results were confirmed in another report.[113]

Bromocriptine and pergolide, drugs with potent dopamine agonist activity, are the treatments of choice for prolactinomas. [30,114] Bromocriptine doses ranging from 5 to 20 mg per day, with a gradual increase in dosage to avoid such initial side effects as nausea and orthostatic hypotension, frequently are sufficient to correct the hyperprolactinemia and to cause regression of the tumor. Pergolide is tenfold more potent than bromocriptine, with the advantage of once-daily dosing and reduced cost. Newer ergot-dopamine agonists, such as cabergoline, can be given twice weekly, are as efficacious as the other dopamine agonists, but are much more expensive. Years of treatment with dopamine agonists may be required for permanent cure. Bromocriptine, usually at higher doses (up to 25 mg per day), occasionally is helpful in the treatment of somatotropinomas and chromophobe adenomas.[53,77,114,115]

Pituitary adenomas and craniopharyngiomas usually are radioresistant. However, radiotherapy in doses as high as 5,000 cGy divided into 200-cGy fractions has been applied for the treatment of corticotropinomas and somatotropinomas if surgery has been unsuccessful. [112,113,116,117] Radiotherapy of craniopharyngiomas after subtotal excision may decrease the incidence of recurrences.[92,118]

Corticotropinomas respond relatively well to irradiation, with approximately 70% to 80% of these lesions in children being cured by 1 to 2 years after therapy. [116] Nonendocrine complications of radiotherapy have included an increase in second tumors within the radiation field, visual impairment, and problems with memory. The use of multiportal fractionated irradiation of approximately 50 Gy over a 5-week period has reduced these complications.

An alternative to radiotherapy is bilateral adrenalectomy. Patients then are committed to lifelong glucocorticoid and mineralocorticoid replacement. Hydrocortisone (12 to 15 mg per $m^2$ per day) and 9a-fluorocortisone (50 to 150 mg per day) are recommended. In approximately 15% of patients so treated, Nelson's syndrome (i.e., pituitary ACTH-secreting macroadenoma and hyperpigmentation) develops within 10 years after adrenalectomy. [119] Likely, the so-called Nelson's syndrome, first described in 1958 at a time when radiologic imaging modalities and neurosurgical pituitary skills were not as well developed as they are today, merely represents the term for a less well resected and subsequently recurrent or progressed pituitary adenoma. If the visual system is threatened, transsphenoidal surgery or radiotherapy is indicated.

Adrenolytic agents, such as o,p'-DDD (mitotane), or steroidogenesis enzyme inhibitors, such as aminoglutethimide, metyrapone, trilostane, and ketoconazole, may be employed to control hypercortisolism.[120,121,122,123 and 124] Patients tolerate most of these drugs poorly. However, ketoconazole (10 to 15 mg per kg per day) is relatively well tolerated and frequently is used. This drug has some hepatotoxicity, and liver function should be monitored in patients eceiving it. Skin rash is a common complication of aminoglutethimide treatment.[125]

When pituitary tumors are associated with pituitary hormone deficiencies, replacement treatments should be instituted. The deficiencies may develop after surgery or years after radiotherapy. The replacement treatments include GH (0.3 mg per kg per week subcutaneously divided in daily doses) for GH deficiency; hydrocortisone (12 to 15 mg per $m^2$ per day orally or 30 to 100 mg per $m^2$ per day orally or parenterally during significant stress) for adrenal insufficiency; and $T_4$ (1.6 µg per kg per day orally) for hypothyroidism. Testosterone enanthate (200 mg intramuscularly every 2 weeks) and one of the estradiol and progestin combinations may be employed for the treatment of male and female hypogonadism, respectively. Desmopressin (DDAVP; 0.1 mL intranasally, as needed) is the treatment of choice for diabetes insipidus. AVP (Pitressin) is available for subcutaneous administration. (Several sources providing more information on hormonal replacement therapies in children are provided in the reference list.[1,11,12])

The prognosis for patients with pituitary tumors generally is good. These tumors are benign, although some have a tendency to invade adjacent structures. In a series of pediatric patients with non–ACTH-secreting pituitary adenomas, those with microadenomas had a 70% operative cure rate and a 65% long-term cure rate. The recurrence rate for microadenomas was 25%. Macroadenoma patients had a 33% operative cure rate, a 55% long-term cure rate, and a recurrence rate of 33%. In the same series, macroadenoma patients required more aggressive adjuvant therapy and had higher rates of postsurgical hypopituitarism. [126] Frequently, complete removal of macroadenomas or craniopharyngiomas with suprasellar extensions is difficult and requires adjuvant radiotherapy. Treatment of these tumors by surgery, irradiation, or both methods may add to the endocrine morbidity. Monitoring of visual function is crucial.

Surgery or irradiation rarely is needed for hypothalamic hamartomas. The early identification of these tumors is necessary for timely treatment of the associated central precocious puberty with a gonadotropin-releasing hormone analog. [40,127]

## THYROID TUMORS

Thyroid tumors are divided into adenomas and carcinomas (Table 37-1).[128,129,130 and 131] Both types can secrete hormones. Medullary carcinoma, a form of thyroid carcinoma that arises from the parafollicular (C) cells of the thyroid, produces and frequently secretes calcitonin, which is a tumor marker for this type of cancer. Differentiated thyroid carcinomas usually secrete thyroglobulin. Thyroglobulin secretion, however, also occurs in hyperplastic benign thyroid conditions and depends on the amount of thyroid tissue, as it can be elevated in large goiters.

Thyroid carcinomas are malignant tumors. Despite their histologic appearance, however, the clinical course frequently is relatively benign, with an excellent survival rate when treated appropriately and in a timely manner.

Thyroid nodules can occur spontaneously or in association with hyperstimulation of the thyroid by TSH or thyroid-stimulating immunoglobulins. Nodules associated with overstimulation of the thyroid tissue are seen in iodopenic goiter, Hashimoto's thyroiditis, Graves' disease, or mixed states of Graves' disease and Hashimoto's thyroiditis. In clinically evident nodules, only 1 nodule in 20 may harbor thyroid cancer in adults, but the ratio in children is much higher. In children, thyroid carcinoma is found in approximately 40% of thyroid nodules that come to surgical exploration. Benign lesions that cause nodule formation, such as multinodular or colloid goiters, occur more often in adults than in children.[132,133,134,135,136,137,138,139,140,141 and 142]

### Epidemiology and Genetics

Thyroid carcinomas in childhood represent approximately 1.5% of all tumors before the age of 15 years and 7.0% of the tumors of the head and neck. Two-thirds of pediatric thyroid carcinomas occur in girls, with a peak incidence between ages 7 and 12. [143,144,145,146,147,148,149,150,151,152,153,154 and 155] The causative role of neck irradiation in the development of thyroid cancer has been well established.[156,157,159,160,161,162,163 and 164]

The tumorigenic effect of radiation is more severe in a child's thyroid than in an adult's. This has been demonstrated in epidemiologic studies from the Marshall Islanders, survivors of the atomic blasts in Hiroshima and Nagasaki, and the Belarus-Chernobyl area. [129] Doses exceeding 150 cGy exert a carcinogenic effect, with an average latency of 7 years between irradiation and the appearance of thyroid carcinoma. The incidence of thyroid cancer has decreased since the mid-1960s, when widespread application of radiotherapy to the neck was discontinued. The radiation was given in the neonatal period for enlarged thymus and later for tonsillitis, adenoid hypertrophy, pharyngitis, and skin diseases of the face and neck. Therapeutic irradiation and chemotherapy resulted in a 53-fold increased risk of thyroid carcinoma in patients who had survived 2 or more years after the diagnosis of a cancer in childhood. Children who received 6 cGy to the thyroid during irradiation for tinea capitis had four times the rate of thyroid cancer as did controls. [165]

The incidence of thyroid cancer after atomic fallout increases, as experience with the Chernobyl nuclear accident in the former Soviet Union has shown. [129,166] The

latter event caused a large increase in the number of new cases of this cancer in central Europe only 4 years after the accident occurred in 1990. The cancers developed by these children were unusually aggressive and carried frequent *RET*/PTC rearrangements. The same rearrangements have been described in inhabitants of New Caledonia, a French territory in the Pacific located between Australia and Fiji, and in children and young adults evaluated in Washington, DC. [167,168 and 169] Children who were younger than age 8 and lived in the resettlement zone around the Chernobyl area received an average thyroid radiation dose of 4.7 Gy, as opposed to adults, who received 1.6 Gy. Effects of radioactive iodine in these iodopenic individuals took place on grounds of endemic iodopenic goiter, which is characteristic for the Belarus region and surroundings. The significant increase of thyroid nodule pathology in children of the Republic of Belarus constituted adenoma, nodular goiter, and especially carcinoma. The gender ratio of the patients with papillary thyroid carcinomas was 1.0:1.2. In addition to the study of cancer, a study among atomic bomb survivors documented increased incidence of adenomas, cysts, and autoimmune hypothyroidism.[170]

In addition to diet (e.g., iodine load), genetic factors appear to play a significant role in the pathogenesis of sporadic thyroid cancer. Some reports have cited increased incidence of thyroid cancer in children with the Pendred, Gardner, Cowden, and familial polyposis syndromes and in those with Carney complex. [171,172] Familial incidence of papillary thyroid cancer also has been reported.

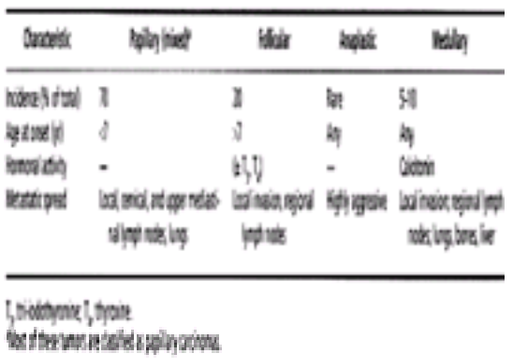
An association appears to exist between disorders of the immune system involving the thyroid gland and thyroid cancers, as reports of papillary and follicular cancer in children with Hashimoto's thyroiditis and lymphoma of the thyroid have suggested. Patients with Hashimoto's thyroiditis have been postulated to have an inherited isolated defect in immunoregulation containing an organ-specific defect in suppressor T-cell function. Such a defect adversely affects immune surveillance, and these subjects may be rendered susceptible to the development of Hashimoto's thyroiditis, thyroid neoplasia, or both conditions. Other factors, such as serum TSH, TSH receptor–activating mutations, oncogene activation, and local growth factors (e.g., IGF-1, transforming growth factor-a, epidermal growth factor) also may play a role in thyroid oncogenesis.[131,132,133,134,135 and 136]

Medullary carcinomas of the thyroid frequently are familial. They can be isolated (i.e., familial medullary thyroid carcinoma) or associated with pheochromocytoma in MEN IIA and MEN IIB. These familial cancer syndromes are transmitted in an autosomal dominant manner.[6,27,173,174 and 175] At least 30% of medullary carcinomas are of the familial type; the remainder occur sporadically. Mutations of the RET proto-oncogene, the gene responsible for the MEN IIA and MEN IIB syndromes, have been found in sporadic and familial medullary thyroid cancers. [176,177,178,179,180 and 181]

Hyperthyroidism, goiter, and benign nonfunctioning nodules frequently have been associated with the McCune-Albright syndrome. Activating mutations of the Gsa subunit of the G proteins that mediate thyrotropic action have been found in such patients. An increased incidence of thyroid nodules, and possibly cancer, also have been reported in patients with Carney complex.[182,183]

**Pathology**

Most thyroid carcinomas in childhood are differentiated tumors. [129,157,158 and 159,184,185 and 186] Rarely, undifferentiated (anaplastic) carcinomas, Hürthle cell carcinomas, or lymphomas are found. Metastases to the thyroid, which in adults derive most commonly from a primary renal, breast, or lung cancer, have not been reported in children. In principle, two types of differentiated thyroid carcinomas derive from the follicular epithelium: papillary and follicular carcinomas. Each cancer may be subdivided further into variants that give rise to such combinations as the follicular variant of papillary thyroid carcinoma, which has been observed in 21% of tumors that occurred in patients from the Belarus-Chernobyl region. [129] The relevant features of the various pathologic subtypes are listed in Table 37-4.




[, thioldihydrotyrosine; ], thyroxine.
*Most of these tumors are classified as papillary carcinoma.

**TABLE 37-4. TYPES OF THYROID CARCINOMA IN CHILDREN AND ADOLESCENTS**

Papillary carcinoma may occur in disseminated neoplastic foci (multifocal papillary thyroid carcinoma) in the gland. [128] The epithelial cells are arranged in the form of papillae containing fibrous tissue and vessels. Approximately 50% of papillary thyroid tumors contain psammoma bodies, a structure that forms when calcium salts are deposited on the dying cells of infarcted papillae. The bodies are found in the cores of papillae or in tumor stroma. Tumor cell nuclei are larger and more elongated than those of normal follicular epithelial cells and contain characteristic intranuclear inclusions. “Multifocality” and lymph node metastases are caused by intraglandular lymphatic spread. This form of thyroid cancer makes up 90% of thyroid cancers in children. [129,184] Follicular carcinoma is characterized by adenomatous, follicular formations of cells.[128] Capsular invasion or vascular invasion (or both) distinguishes this tumor from ordinary benign follicular adenoma. Metastatic spread in this cancer often occurs through the blood stream. It occurs predominantly in older children.

Anaplastic (i.e., giant- and spindle-cell) carcinomas are extremely rare in children. They are characteristically undifferentiated, rapidly growing tumors. [128,129 and 130] Medullary carcinomas account for approximately 5% of all thyroid carcinomas. They are solid tumors composed of islets of regularly sized cells with abundant granular cytoplasm. C-cell hyperplasia usually precedes medullary thyroid carcinoma when it occurs within a familial syndrome. [187] The stroma contains abundant fibrotic tissue and various quantities of amyloid-like substance. [128,173]

**Patterns of Spread**

Local cervical and upper mediastinal lymph node involvement by papillary cancer occurs in more than 50% of patients without necessarily implying a poorer prognosis, especially in children. [129,130,143,144] The most common site for distant metastases is the lung and upper mediastinum, and at least 20% of children with papillary thyroid cancer have pulmonary metastases at the time of diagnosis. Bone metastases from papillary cancers are uncommon, as are metastases to any site below the diaphragm. These tumors typically grow slowly.

Follicular carcinomas may be locally invasive but metastasize to regional lymph nodes far less commonly than do papillary cancers. However, they are more likely to spread to bone and the lungs. Follicular carcinomas are more likely to be hypersecretory and functional than are papillary cancers and may produce $T_4$, $T_3$, or both.[159]

Anaplastic carcinomas of the thyroid are extremely malignant. They usually evolve from a previously diagnosed papillary cancer or long-standing, preexisting goiter. The explosive growth of this type of carcinoma may be accompanied by hypercalcemia.

Medullary carcinomas of the thyroid can invade locally or metastasize into the regional lymph nodes. Pulmonary, bone, brain, and liver metastases also can be seen.[26,175]

**Clinical Presentation**

The most common presenting complaint in children is anterior cervical adenopathy. The cervical mass may be discrete and may have been neglected for years before the physician decides to obtain a biopsy specimen. Frequently, the mass has been diagnosed as lymphadenitis or as a congenital branchial cyst. The second most common symptom is a firm, palpable thyroid nodule, isolated or associated with a cervical lymph node. The combination of adenopathy and a thyroid nodule is found

in approximately 50% of cases. Patients are predominantly euthyroid; hyperthyroidism occurs rarely. [129,159]

Patients with thyroid carcinoma can present with a solitary nodule or multiple discrete nodules. The risk of malignancy was thought to be lower in the latter case, although recent reports are debating this observation. Every child with abnormal thyroid gland examination findings deserves a thorough workup, because thyroid nodules are uncommon at a young age. A study of 4,819 healthy children found only 22 patients with a solitary thyroid nodule. Fourteen patients underwent surgical exploration, and two were found to have papillary carcinoma. [184,188]

Obtaining affected patients' histories should include questions on a history of external irradiation to the head and neck, goitrogen ingestion, and the presence of local or systemic symptoms, such as hoarseness or dysphagia. Rapid painless growth of a nodule suggests carcinoma, whereas tenderness indicates hemorrhage in a cyst or an inflammatory process. Physical examination should be directed also to the recognition of signs of a possible genetic syndrome, such as familial medullary thyroid carcinoma, MEN IIA, or MEN IIB. A medullary carcinoma within the context of MEN IIB should be suspected in patients with a thyroid nodule, marfanoid body habitus, and multiple mucosal neuromas, appearing as whitish nodules on the tongue, the lips, and the conjunctivae. At times, rapidly developing dysphagia may result from an ectopic thyroid gland. [189]

**Diagnosis**

In children with thyroid tumors, tests of thyroid function usually confirm euthyroidism. Rarely, hyperthyroidism is seen. Serum concentrations of thyroglobulin can be elevated in patients with thyroid carcinoma but may be elevated also in patients with benign thyroid disorders. This test is useful, however, for postoperatively monitoring patients with carcinoma.

Thyroid scintigrams performed with $^{123}I$ or $^{99m}Tc$ usually show parenchyma with normal uptake and one or more “cold” or hypofunctioning nodules ( Fig. 37-3). Rarely, “hot” or hyperfunctioning nodules are seen. Discrepancies between scintigrams taken with $^{123}I$ and $^{99m}Tc$ can occur. Because a carcinoma can appear hot with $^{99m}Tc$ and cold with $^{123}I$, patients with the former finding require repeat scanning with radioactive iodine. Octreotide scintigraphy appears useful for the detection of medullary thyroid carcinoma.

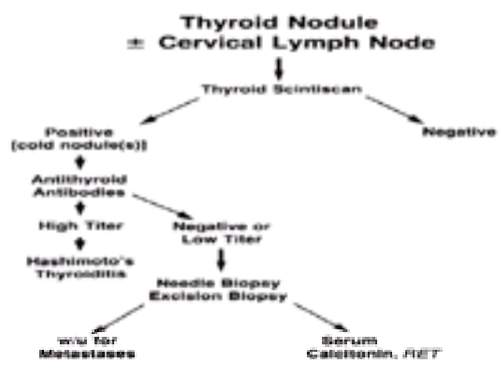



**FIGURE 37-3.** A proposed flow diagram for the diagnostic evaluation of thyroid nodules in children and adolescents. w/u, workup.

Very small nodules may not be imaged by one or both of the foregoing techniques. Ultrasonographic examination of the thyroid gland in pediatric patients provides an accurate means of assessing thyroid size and the presence and size of thyroid nodules. This study is recommended routinely as one of the first studies that should be obtained in the evaluation of a solitary thyroid nodule in a child. Chest radiographs or chest computed tomography (CT) or MRI should be obtained to exclude metastatic spread.[190]

Very high titers of antithyroglobulin or antimicrosomal antibodies render the diagnosis of Hashimoto's thyroiditis likely, but low titers are not helpful diagnostically ( Fig. 37-3). A basal serum calcitonin assay can be helpful in pointing toward or away from medullary carcinoma. If the result is equivocal or if suspicion of medullary carcinoma is high, a stimulation test using pentagastrin or calcium infusion should be performed. Recently, calcitonin stimulation also was obtained by administration of omeprazole.[191] A stimulated calcitonin concentration three times higher than the upper limit of normal suggests medullary cancer or C-cell hyperplasia, a precancerous condition.[187]

The best single diagnostic test for the diagnosis of thyroid nodules is fine-needle aspiration biopsy. [192] If the specimen obtained by this procedure is unsatisfactory, an excisional biopsy of the enlarged cervical lymph node or of the isolated thyroid nodule may be required. For younger children, an open excisional or core biopsy, rather than a fine-needle aspiration biopsy, is recommended.

**Treatment**

***Surgery***

Surgery is the treatment of choice for thyroid carcinoma. [157,159,184,185] and [186,193,194,195] and [196] Total thyroidectomy is recommended but should be carried out by an experienced endocrine surgeon to avoid complications, such as recurrent laryngeal nerve injury and postoperative hypoparathyroidism. Performance of a total thyroidectomy facilitates subsequent radioactive ablation of the thyroid remnant. This positively affects disease-free survival, as shown by univariate and multivariate analyses.[129,186,195,196] Similarly, lymph node dissection should be performed if lymph node involvement is documented preoperatively or intraoperatively. During surgery, frozen sections of sampled tissue can be obtained to assist in deciding on the extent of surgical removal, but clinical surgical judgment is of paramount importance. Postoperatively, all patients are given replacement doses of levothyroxine ($LT_4$) (2.2 mg per kg per day) to suppress TSH, thereby eliminating the growth-promoting effects of this hormone on the tumor. Side effects of this suppressive treatment, which causes mild iatrogenic hyperthyroidism, include vascular headaches in children from ages 8 to 20, insomnia, attention deficits that may persist over the long term, and effects on skeletal maturation and calcification with subsequent osteopenia.[129,130,197]

***Radioiodine***

After surgical treatment of differentiated thyroid cancer, the decision of whether to administer $^{131}I$ must be made. Most differentiated thyroid tumors accumulate $^{131}I$, which provides high levels of radiation to the cancer cells. Cancer outside the confines of the thyroid is a definite indication for such therapy. Because at the time of diagnosis more than 20% of affected children have lung metastases that may or may not be apparent on chest radiographs, $^{131}I$ therapy generally is recommended for children. Before and 5 days after $^{131}I$ therapy, a diagnostic whole-body radioactive scan should be performed. Therapeutic doses of $^{131}I$ for metastases are administered only after $^{131}I$ dosimetry and thyroid ablation therapy have been performed. [130,198,199,200,201,202,203] and [204] If no metastatic disease is seen outside the thyroid bed, $^{131}I$-induced ablation of any significant thyroid remnant is required to eliminate thyroid function. This also facilitates the use of thyroglobulin as a tumor marker for recurrent disease during periodic follow-up under hypothyroid conditions achieved by $LT_4$ withdrawal.

$^{131}I$ doses for ablation vary from center to center. Usually, approximately 29 mCi are sufficient for thyroid bed destruction in adults, but most authorities recommend ablation with 30 to 100 mCi for low-risk cases. In the past, much higher doses have been used. As children are more sensitive to radiation than adults and are at risk for developing secondary cancers for a longer projected life expectancy after radioiodine treatment, 29 mCi should be sufficient for ablation. On the other hand, in the presence of lymph node or pulmonary metastases, much higher doses of $^{131}I$ are needed. TSH stimulates tumor uptake of $^{131}I$ and enhances the probability of its detection by diagnostic scanning and its therapeutic effectiveness. Recombinant TSH has proven useful in detecting and treating residual tumor and tumor metastases.[205,206]

After preparation (e.g., avoidance of iodized substances, intravenous radiographic contrast preparations) and usually 4 weeks after surgery or 6 weeks after

discontinuation of $T_4$ replacement therapy, affected patients are given a standard thyroid-ablation dose of 29 mCi of $^{131}I$ and are placed on $T_4$ replacement. During the hypothyroid preparatory period, patients are allowed to take $T_3$ (Cytomel) to ameliorate symptoms from hypothyroidism. Cytomel must be stopped in a patient 2 weeks before scanning of that patient is undertaken. Six months after thyroid ablation, standard scanning with 1 to 5 mCi of $^{131}I$ is repeated under hypothyroid conditions. If less than 0.3% of the dose is found in the thyroid bed at 48 hours, thyroid ablation has been successful. This outcome occurs in approximately 80% of cases. Between follow-up evaluations, patients are maintained on thyroid hormone suppressive therapy (2.2 µg per kg per day).

$^{131}I$ therapy for metastatic disease is administered after successful thyroid ablation. Standard fixed therapeutic doses (150 to 200 mCi) may be given every 6 months. Alternatively, the dose may be calculated by previous dosimetry, with excellent results. More than one-half of patients with metastatic disease are rendered free of disease by $^{131}I$ therapy. Most cures are obtained after one or two therapeutic doses, although some patients may require more.

The side effects of $^{131}I$ include transient bone marrow suppression (e.g., decrease of circulating leukocytes and platelets with a nadir at approximately 6 weeks), nausea and vomiting, sialadenitis, pain in metastatic deposits, pulmonary fibrosis, and leukemia. [201] The sialadenitis may be permanent and leads to deterioration of the teeth. Azoospermia and decreased fertility may occur. [207] With use of pretreatment dosimetry for metastatic disease, serious complications, such as radiation-induced pulmonary fibrosis and leukemia, are thought to occur less frequently.

***Other Treatment***

Chemotherapy or external-beam irradiation for metastatic differentiated and anaplastic thyroid cancer have been disappointing. [159,208] Doxorubicin (Adriamycin) is the only proven active single agent, and its results often are temporary. Local control of anaplastic cancer of the thyroid has been rare until the use of a combination of low-dose (10 mg per $m^2$ per week) doxorubicin and external-beam irradiation (200-cGy fractions; total dose, 5,000 cGy). [209] This combination has been successful also in local control of bulky recurrences of differentiated cancer. [210]

**Prognosis**

The prognosis for patients with differentiated thyroid carcinoma generally is good. The survival of children and adolescents up to 1970 was approximately 82% at 20 years but, in series published since 1981, survival rates in excess of 90% have been reported. The presence of distant metastases does not necessarily predict a poor prognosis. Patients should not be overtreated with extensive surgery, $^{131}I$, or external-beam irradiation. Furthermore, $^{131}I$ successive therapies in persistent metastatic disease should be used judiciously to avoid high cumulative $^{131}I$ dose exposures, which in children and adolescents may have a high risk for the emergence of secondary neoplasias, such as leukemias.

Frequent monitoring of patients with differentiated thyroid cancer or medullary carcinoma of the thyroid is important. Physical examination, chest radiographs, and at least annual measurement of plasma concentrations of thyroglobulin and calcitonin or carcinoembryonic antigen are important. [211,212 and 213] If the serum thyroglobulin level becomes detectable or rises after completion of surgery and radioiodine ablative therapy while a patient is on TSH suppressive therapy with $LT_4$, repeat total-body scanning with different imaging modalities is indicated under hypothyroid conditions or $LT_4$ withdrawal.

Patients with thyroid cancer may survive for many years with good quality of life. [159,214] However, repeat surgery, $^{131}I$, or both may be necessary between long asymptomatic intervals.

## PARATHYROID TUMORS

Parathyroid tumors include adenomas, usually found in one or, rarely, two glands; hyperplasia, usually affecting all four glands; and carcinomas. [4,5,215,216,217,218,219,220,221,222,223,224,225 and 226] Adenomas account for approximately 80% of parathyroid tumors, hyperplasia accounts for 20%, and carcinoma accounts for only a few cases. Parathyroid tumors secrete parathyroid hormone (PTH), which is responsible for the syndrome of primary hyperparathyroidism.

Parathyroid hyperplasia or adenomatous changes also can occur in conditions characterized by chronic hypocalcemia, including hypovitaminosis D, intestinal malabsorption of calcium, PTH resistance, and renal insufficiency. [1,2 and 3,227,228 and 229] Parathyroid hyperplasia can be found also in X-linked hypophosphatemic rickets [230,231] and familial hypocalciuric hypercalcemia, a benign condition caused by mutations in the calcium sensor gene and requiring no parathyroid surgery. [232,233 and 234]

**Epidemiology and Genetics**

Primary parathyroid tumors in childhood are rare. They can occur in young persons at any age, from neonates to young adults. Most cases are not hereditary. However, familial parathyroid adenoma and hyperplastic states exist. [1,2 and 3,222] In MEN I, parathyroid disease is the most frequently occurring component of the syndrome. Even in the absence of a tumor, all of an affected individual's parathyroid glands are hypercellular in this condition. The gene responsible for MEN I has been identified on chromosome 11q13. [235] This gene acts as a tumor suppressor gene, with loss of the wild-type copy in MEN I–associated tumors. Interestingly, clonal allelic losses of chromosome 11q13 also have been observed in 25% of sporadic parathyroid adenomas, as well as a number of non–MEN I–associated, GH-producing pituitary and other tumors. [236,241]

A subset of sporadic parathyroid adenomas contains a clonal genomic DNA rearrangement involving chromosome 11 and leading to the fusion of an oncogene named *PRAD1* (located at 11q13) with a part of the PTH gene (located at 11p15). This pericentric inversion leads to overexpression of the *PRAD1* transcript and parathyroid tumorigenesis in a way similar to that described for the *bcl-2* oncogene in B-cell lymphomas. [242] Hyperparathyroidism occurs in only a minority (approximately 25%) of patients with MEN IIA and is characterized by multiglandular involvement. Mutations of the RET proto-oncogene, the gene responsible for MEN IIA and MEN IIB, have not been described in sporadic parathyroid tumors. The germline *RET* mutations in patients with MEN IIA have been suggested to cause mild parathyroid hyperplasia but require the presence of additional oncogenic hits in a given cell to provoke the development of clinically significant, monoclonal tumors. In patients with MEN IIB and mutations of the RET proto-oncogene but at a different site of the gene (exon 16), hyperparathyroidism is absent. [243]

Other genetic conditions also are associated with parathyroid hyperplasia and adenoma formation. Familial isolated hyperparathyroidism has been described in several kindreds containing an inordinate number of affected members without other endocrine manifestations. Several of these patients developed parathyroid cancer. Investigators are working to identify the gene responsible for this disorder, known as *HRPT1*. [244]

The gene for the hereditary hyperparathyroidism–jaw tumor syndrome, called *HRPT2* and considered to be an important endocrine tumor gene, has been mapped to the long arm of chromosome 1 (1q21-q31). [245,246,247,248 and 249] Familial hypocalciuric hypercalcemia is also called *familial benign hypercalcemia* and is an autosomal dominant condition in which patients excrete strikingly less calcium in their urine than do other individuals with an equivalently elevated serum calcium. In most families with familial hypocalciuric hypercalcemia, linkage was demonstrated to the G protein–coupled calcium sensor gene, *PCAR1*. Heterozygotes for mutations of this gene appear to have reduced calcium-sensing activity, with resultant activation of PTH secretion and increased renal tubular calcium reabsorption characteristic of the disease. Homozygotes or compound heterozygotes for mutations of the *PCAR1* gene present at birth with severe neonatal hyperparathyroidism due to parathyroid hyperplasia. [234]

**Pathology**

Several pathologic variants have been described in the parathyroid glands of patients with primary hyperparathyroidism. [5,221,222 and 223,227,228,229,230,231,232,233 and 234,242,243,244 and 245,250] The predominant cell in parathyroid adenomas and hyperplasia is the chief cell. Rarely, adenomas composed of oxyphil cells or a mixed population of chief and oxyphil cells are found. The normal parathyroid gland contains as much as 50% fat, but adenomatous or hyperplastic glands contain little or no fat. Classically, a capsule and a compressed rim of normal tissue are seen. In a small percentage of cases, hyperplasia of the clear cell, a variant of the chief cell, is observed.

In parathyroid carcinomas, the tumor is larger than adenomatous or hyperplastic parathyroid glands. [217,219] Histologic examination reveals infiltration of the capsule and blood vessels and mitoses. The slow-growing carcinomas of the parathyroid glands spread locally to the lymphatics. Distant hematogenous metastases are

located in the lung, liver, and bone.

### Clinical Presentation

Primary hyperparathyroidism can be associated with asymptomatic hypercalcemia, fortuitously diagnosed during an electrolyte check, or with the hypercalcemic syndrome, which may manifest as polydipsia, polyuria, mental confusion, pruritus, headache, keratitis petrificans, band keratitis, and disseminated calcifications. [1,2] and [3,250] Bone pain may occur with demineralization and resorption cysts of the phalanges, subperiosteal zones, and lamina dura in the dental alveoli, skeletal deformations, or fractures.

Osteitis fibrosa cystica describes the combination of these lesions, with characteristic brown tumors of the jaws, skull, clavicles, and other areas of the skeleton. Renal involvement with nephrolithiasis and nephrocalcinosis most commonly develops, as may gastric ulcers and pancreatitis.

### Diagnosis

The diagnosis is confirmed by demonstrating hypercalcemia, hypophosphatemia, elevation of serum alkaline phosphatase, and inappropriately high serum concentrations of PTH that cannot be suppressed by infusion of calcium. [1,2] and [3,251] Impaired renal function may manifest as alkalosis, AVP resistance, and sodium wasting. Proximal muscle weakness results from muscle atrophy that is more prominent in type II fibers. Electrocardiography shows shortening of the QT interval. Because the length of the QT interval varies considerably with cardiac rate in children, a correction for rate should be applied. Hypertension is observed in 20% to 60% of patients with severe hyperparathyroidism.

Bone radiographs may reveal characteristic lesions of osteitis fibrosa cystica and findings compatible with rickets. Nephrocalcinosis and nephrolithiasis may be evident on abdominal x-ray films or ultrasonographic or CT scans of the kidneys. Demineralization around the teeth is evidenced as loss of the lamina dura. Ectopic calcifications also may be seen.

Sometimes, adenomas of the parathyroid glands can be localized preoperatively by palpation, radiographs of the esophagus (showing a deviation as a result of impingement by a parathyroid adenoma), ultrasonographic and CT imaging, arteriography, and selective venous catheterization for determination of differences in plasma PTH concentrations. Radioactive thallium-technetium subtractive scintigraphy is the standard localization technique in cases requiring a second surgical exploration. However, in general, locating an excellent endocrine surgeon is of paramount importance. [252,253] and [254]

### Treatment and Prognosis

Treatment of parathyroid adenomas and hyperplasia (primary and tertiary hyperparathyroidism) consists of surgical removal of the afflicted parathyroid gland(s) after careful exploration of the cervical region. [5,255,256,257] and [258] The medical management of hypercalcemia is based on maintaining good hydration, increasing urine calcium excretion, and diminishing bone resorption. Hydration with saline (up to 4 L per day for a full-grown adolescent, proportionally less for younger children) and the administration of furosemide (1 mg per kg three times daily) increase renal calcium excretion. Oral phosphate, glucocorticoids, and diphosphonates have been used in the management of hyperparathyroidism until surgery is undertaken.

If an abnormal parathyroid gland is removed, a second gland should be located and excised. If the size and histology of the second gland are normal, a single adenoma is likely, and further exploration is unnecessary. If hyperplasia is suspected or if the second gland is abnormal, all parathyroid glands should be located, and all but one should be excised totally. The remaining gland should be excised partially. If no glandular abnormalities are found in the cervical region, exploration of the mediastinum should be considered. Intraoperative measurements of PTH after removal of one or more parathyroid glands may assist in determining whether enough tissue that caused hyperparathyroidism before surgery has been removed. [233] Some surgeons advocate transplantation of a portion of some of the parathyroid tissue into the muscle of the forearm to avoid hypoparathyroidism from delayed vascular failure in the portion of the gland left behind. Such a transplanted gland may resume function several weeks or months after surgery or, in rare cases, never. For the postoperative hypoparathyroid period, administration of calcium and vitamin D is necessary to keep the plasma ionized calcium concentration in the low-normal range. [259,260] and [261]

Parathyroid carcinomas are radioresistant, and so surgery is the treatment of choice. Surgical morbidity includes recurrent laryngeal nerve injury and permanent hypoparathyroidism. Failure of initial surgery to cure hyperparathyroidism is an indication for reevaluating the diagnosis and repeating localizing procedures before further surgical exploration. A missed adenoma most likely is located in the mediastinum or in the retroesophageal area of the neck. In the hands of an experienced surgeon, repeat surgery is successful in 90% of cases.

Parathyroid carcinomas occur rarely in children, but they constitute a significant cause of persistent or recurrent primary hyperparathyroidism. [220,224] They tend to be slow-growing and are curable in the early stages by adequate local excision.

The postoperative course of patients treated with parathyroidectomy usually includes transient hypocalcemia for several weeks. If the bone disease has been severe, hypocalcemia may be profound, requiring treatment with calcium and vitamin D. Hypocalcemia in association with hypomagnesemia is termed *hungry bone syndrome* and requires additional magnesium supplementation. Several months may be needed to decide whether permanent hypoparathyroidism has resulted from a compromised blood supply to the remaining parathyroid tissue.

## ADRENOCORTICAL TUMORS

Adrenocortical tumors are divided into adenomas and carcinomas. [54,55,262,263,264,265,266,267,268,269,270,271,272,273,274,275,276,277,278,279,280,281,282] and [283] Adenomas and carcinomas can secrete hormones or can be hormonally inactive. [265,266,267,268,269,270,271,272,273,274,275,276,277,278,279,280,281,282,283,284,285,286,287,288,289,290,291,292,293,294,295] and [296] The hormones secreted include cortisol, aldosterone, androgens, estrogens, and steroid biosynthesis intermediates ( Fig. 37-4). Generally, adenomas are far more efficient than are carcinomas in producing steroid hormones.

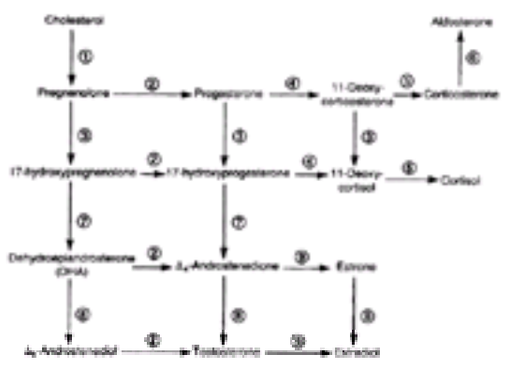



**FIGURE 37-4.** The adrenal steroidosynthesis pathway. Aldosterone, cortisol, androgens, and estrogens are manufactured from cholesterol after a series of enzymatic reactions. Numbers in circles represent enzymes: 1, cholesterol desmolase system; 2, 3-b-hydroxysteroid dehydrogenase-D5, D4-isomerase; 3, 17a-hydroxylase; 4, 21a-hydroxylase; 5, 11b-hydroxylase; 6, corticosterone methyloxidase types I and II; 7, 17,20-desmolase; 8, 17-ketosteroid reductase; 9, aromatase. Enzymatic blocks are common in tumors, resulting in accumulation of one or more steroid precursors in plasma.

Regardless of hormone secretion, adenomas generally are benign, whereas carcinomas are malignant. Adrenal tumors can be found incidentally during adrenal CT or MRI scanning or can be discovered in the course of evaluation for hypercortisolism, hyperaldosteronism, hyperandrogenism, or hyperestrogenism. [297,298] and [299] One or more adrenal adenomas frequently are found during the course of ACTH-dependent Cushing's syndrome (Cushing's disease) and, less frequently, in the ectopic

ACTH secretion syndrome. In this case, they are described as macronodular adrenal hyperplasia and should be differentiated from the micronodular form, primary pigmented nodular adrenocortical disease (PPNAD). PPNAD causes ACTH-independent Cushing's syndrome and most frequently is associated with spotty skin pigmentation (lentigines), other endocrine tumors, and myxomas of the heart, skin, and other sites. The disease is known as *Carney complex*, and in approximately 50% of cases, it is inherited in an autosomal dominant manner.[172] Unilateral or bilateral macronodular adrenal hyperplasia occasionally is ACTH-independent and may cause hyperaldosteronism or, rarely, Cushing's syndrome.

In children older than 5 years, adrenal carcinomas frequently (50% to 60%) secrete cortisol and adrenal androgens and rarely secrete aldosterone, testosterone, or estrogens.[275,276,277,278,279,280,281 and 282] Approximately 40% of adrenocortical carcinomas secrete no active hormones, but inactive steroid precursors (e.g., pregnenolone, 17-hydroxypregnenolone, and 11-deoxycortisol) or their metabolites can be found in the circulation and the urine, respectively. Occasionally, adrenocortical carcinomas secreting deoxycorticosterone or corticosterone cause hypokalemic alkalosis in the absence of hypercortisolism.[279] Generally, hormone-secreting adrenocortical carcinomas are very inefficient in producing active hormones such as cortisol, and nearly 50% of them have attained palpable size by the time they produce an endocrine syndrome. Among children younger than 5 years of age, 95% of the cases present with virilization. A higher incidence of cancers may be noted among relatives of patients with adrenocortical carcinoma.

**Epidemiology and Genetics**

Adrenal adenomas and adrenocortical carcinomas that cause Cushing's syndrome are rare tumors.[54,55] In older children and adolescents, adrenal tumors are responsible for 10% to 20% of cases of Cushing's syndrome but, in children younger than 5 years, they and the less common ectopic ACTH-secreting tumors are together responsible for approximately 80% to 90% of cases of Cushing's syndrome. In a series of 59 children with Cushing's syndrome who were between the ages of 4 and 20 years, four had PPNAD, and two had carcinomas.[109]

The genetics of sporadic adrenal adenomas and carcinomas remain largely unknown, with many genetic alterations representing merely genetic epiphenomena. One-third of adrenocortical carcinomas were found to have mutations of the p53 tumor suppressor gene.[300] Cushing's syndrome in McCune-Albright syndrome is caused by activating mutations of the Gsa subunit. The gene responsible for Carney complex, the single inherited form of adrenal hyperplasia (PPNAD) leading to Cushing's syndrome, was mapped to the short arm of chromosome 2 (2p16) and the long arm of chromosome 17, but its nature remains unknown.[172,301]

Many sporadic adrenal carcinomas demonstrate loss of heterozygosity involving the insulin-like growth factor-2 (IGF-2) locus at the short arm of chromosome 11 (11p13).[302,303] IGF-2,a gene that is paternally imprinted, is overexpressed in these tumors, and adrenal carcinomas are common in a genetic disorder, the Beckwith-Wiedemann syndrome, that has been associated with IGF-2 gene up-regulation and loss of imprinting status. Although it appears certain that growth factors are important in adrenal oncogenesis, that they are the primary genes involved remains doubtful. One study failed to identify oncogenic mutations of the Gsa subunit in adrenal tumors. An autosomal dominant disorder that predisposes to multiple neoplasias, including adrenocortical carcinoma and osteosarcoma, is Li-Fraumeni syndrome, which is caused by germline mutations of the p53 tumor suppressor gene.[304]

**Pathology**

Adrenal adenomas are generally small, encapsulated, steroid-secreting tumors with characteristically increased smooth endoplasmic reticulum and lipid droplets inside the cells.[262,263 and 264] Frequently, few signs of malignancy can be found; however, numerous mitoses and pleomorphism can be seen without capsular invasion. Micronodular adrenal hyperplasia is characterized by the presence of small nodules dispersed in both adrenal glands.[305,306,307 and 308,310] The nodules contain a brown or black pigment. Macroscopically, they give the adrenal cortex a rugged appearance. The internodular parenchyma is hypotrophic or atrophic.

In contrast, adrenocortical carcinomas are large by the time they are discovered.[262,263] They infiltrate neighboring tissues such as the kidney capsule, and they spread locally. Cells frequently are characterized by numerous mitoses, scant cytoplasm, and pleomorphism. Areas of necrosis and hemorrhage within the tumor are common, and such hemorrhage can cause death. At present, malignancy is defined by the “Weiss criteria.”

**Patterns of Spread**

Local spread characterizes adrenocortical carcinomas. By the time they are discovered, 20% of the tumors have spread locally. Tumors spread into the kidneys, the retroperitoneal and peritoneal space, the diaphragm, and the vena cava. Occasionally, the tumor grows up into the right atrium. Intrahepatic spread frequently is observed. Lung and bone metastases also are common.

**Endocrine Manifestations**

The most common endocrine manifestations are virilization and Cushing's syndrome.[54,55,112] Distinguishing this from the other forms of Cushing's syndrome is relatively easy, because the tumor is identified radiologically by CT or MRI scans and no plasma ACTH is detectable because of pituitary ACTH suppression by the elevated plasma cortisol concentration. Adrenal adenomas or carcinomas fail to respond to low or high dexamethasone doses ( Table 37-3).

Other endocrine manifestations include hyperaldosteronism characterized by hypertension, hypokalemic alkalosis, and elevated plasma concentrations and 24-hour urinary excretion of aldosterone or other sodium-retaining corticoids. Hyperandrogenism, characterized by precocious puberty in the male patient or masculinization in the female patient, and hyperestrogenism, associated with feminization and hypogonadism in boys and precocious puberty in girls, may also be seen.

**Diagnosis**

Imaging procedures are the key to the diagnosis of adrenal tumors.[264,299,311,312,313,314,315,316 and 317] Ultrasonography should be the initial imaging modality for children with adrenal masses, followed by CT scanning or MRI. The latter, especially T2-weighted relaxation imaging, is characteristically enhanced as a consequence of the high water content of adrenocortical carcinomas. In the case of micronodular adrenal disease, small rugged adrenal glands may be seen bilaterally, but often the nodules are not visible by any of the aforementioned techniques. A radioactive iodocholesterol scan allows imaging of cortisol-secreting adenomas but not of carcinomas or 50% of adrenal glands with micronodular disease.

Depending on the associated endocrine syndrome—hypercortisolism, hyperaldosteronism, hyperandrogenism, or hyperestrogenism—the diagnosis can be confirmed, respectively, by elevated 24-hour UFC, urinary aldosterone, plasma androgens or urinary 17-ketosteroids, or plasma estrogens. In the rare case of hyperaldosteronism attributable to secretion of a steroid other than aldosterone that has sodium-retaining properties, the plasma concentrations of such steroid intermediates as deoxycorticosterone and corticosterone should be measured ( Fig. 37-4). The measurement of serum 18-hydroxycorticosterone after overnight bedrest is a useful test for differentiating aldosterone-producing adenomas from primary adrenal hyperplasia (i.e., idiopathic hyperaldosteronism).[318]

**Treatment**

The treatment of all primary adrenal tumors is surgical, with laparoscopic removal of the affected adrenal gland.[5,54,55,112,113,280,319,320 and 321] Adrenal adenomas should be removed with the whole ipsilateral adrenal gland. Micronodular adrenal disease (PPNAD) or primary macronodular adrenal disease should also be cured by bilateral adrenalectomy performed laparoscopically. Unilateral adrenalectomy has been performed in a number of patients with primary adrenal hyperplasia (i.e., idiopathic hyperaldosteronism). In these patients, hypertension resolved, and medical treatment or repeat surgery was not needed.

Complete resection of the tumor by laparotomy is the treatment of choice for adrenal carcinoma. If complete resection cannot be achieved, as much of the tumor as possible should be removed. Solitary recurrences or metastases of adrenocortical carcinoma should also be removed surgically. Long-term disease-free status has been produced by complete resection of adrenocortical carcinoma, and long-term remissions have followed surgical resection of hepatic, pulmonary, or cerebral metastases.

If the patient does not have surgically curable disease, therapy with o,p'-DDD (mitotane) usually is initiated. The o,p'-DDD, an adrenocytolytic agent given at maximally tolerated oral doses (up 10 g per $m^2$ per day), ameliorates the endocrine syndrome in approximately two-thirds of patients.[54,55,322,323,324 and 325] Tumor regression or arrest of growth has been observed in as many as one-third of patients. However, mean survival does not appear to be altered, although some patients

with unresectable carcinomas have achieved long-term survival. The side effects include nausea, vomiting, diarrhea, skin reactions, and neurologic manifestations, primarily lethargy, somnolence, dizziness, and muscle weakness. Such other chemotherapeutic agents as cisplatin, 5-fluorouracil, and etoposide may be useful.

Occasionally, for the correction of hypercortisolism, steroid synthesis inhibitors (i.e., aminoglutethimide, metyrapone, trilostane, ketoconazole) or glucocorticoid antagonists [mifepristone (RU-486)] are required. [326,327,328,329 and 330] Patient staking o,p'-DDD may develop hypoaldosteronism or hypocortisolism, and fludrocortisone or hydrocortisone should be added as needed. Radiotherapy occasionally is helpful for palliation of metastases. Recently, a new interventional technique, radiofrequency ablation, has been used in a few patients. [331,332]

After removal of an autonomous adrenal adenoma or carcinoma, a period of adrenal insufficiency ensues, during which glucocorticoids must be replaced. [54,55] This abnormality of the hypothalamic-pituitary-adrenal axis can last as long as 1 year or occasionally longer. Through the first 2 postoperative days, a total of 100 mg of hydrocortisone per day or its equivalent is given intravenously. New guidelines for glucocorticoid coverage during stress periods subdivide stressors into different stress stages—for instance, considering cholecystectomy only a moderate stressor as compared to coronary artery bypass surgery. Each stress stage is assigned a different glucocorticoid coverage dose—for instance, up to 50 mg of hydrocortisone for a single day for minor stress, up to 100 mg per day for 1 to 2 days for moderate stress, and up to 150 mg per day for 2 to 3 days for severe stress [e.g., cardiac surgery such as coronary artery bypass graft (CABG)]. [333] Postoperatively, oral replacement doses of hydrocortisone (12 to 15 mg per $m^2$ per day) then are initiated. Patients often complain of weakness at these doses. This regimen is maintained until the patient is tested 3 months later with a short ACTH stimulation test [cosyntropin (Cortrosyn), 10 mg per kg intravenously, with serum cortisol measured at 1 hour]. If the response to this test is normal (plasma cortisol in excess of 18 µg per dL), an attempt is made to discontinue hydrocortisone therapy. If the result is subnormal, the therapy is continued for another 2 months, and the test is repeated. During the period of adrenal insufficiency, patients should be given extra glucocorticoids in the form of replacement when stressed. During minor stress (e.g., febrile illness), they should double the daily dose for 2 or 3 days. During major stress (e.g., trauma, surgery), they can be given up to ten times the replacement dose for 2 or 3 days. All patients should wear medical alert badges indicating that they are receiving glucocorticoid replacement.

### Prognosis

The prognosis for patients with primary adrenal adenomas and micronodular adrenal disease is excellent. Patients with idiopathic hyperaldosteronism should be followed closely after unilateral adrenalectomy for recurrence of hypertension. The prognosis of adrenal carcinoma is generally poor, with a mean survival of approximately 18 months. Highly aggressive tumors can progress rapidly in a few months. With aggressive surgical therapy, the mean survival increases to 48 months, and survival as long as 10 years has been described for some patients receiving vigilant monitoring and aggressive surgery for local recurrences or metastases. Cures have been achieved for patients undergoing operation early, while the tumor was still encapsulated.

## CHROMAFFIN CELL TUMORS

Tumors of the adrenal medulla arise from chromaffin cells and are called *pheochromocytomas*. Tumors that do not primarily develop from the chromaffin cells of the adrenal medulla but rather from extra-adrenal sympathetic nervous system structures are known as *paragangliomas*. Pheochromocytomas and paragangliomas commonly synthesize and secrete catecholamines, including epinephrine, norepinephrine, dopamine, metanephrine, and normetanephrine. [4,5,29,334,335,336 and 337] The neuroendocrine origin of pheochromocytomas is underscored by positive immunostaining for chromogranin A and synaptophysin and granules visible by electron microscopy.

### Epidemiology and Genetics

Pheochromocytomas are rare tumors. In childhood, these tumors are predominantly diagnosed between the ages of 6 and 15 years with a slight predominance in boys.[338,339,340,341 and 342,367] Malignancy in these tumors is established by the presence of distant metastases. Approximately 10% of pheochromocytomas that occur intra-adrenally are reported to be malignant, as opposed to 40% of those with extra-adrenal location of the primary tumor. Pheochromocytomas most often occur as sporadic tumors but develop also as part of a hereditary syndrome. Most familial pheochromocytomas can be subdivided into the following groups: MEN IIA, MEN IIB, VHL disease, and NF-1.[26,29,343,344] Paragangliomas (tumors that develop in extra-adrenal structures of the sympathetic nervous system such as the organ of Zuckerkandl) also can occur in a familial syndrome that has been linked to chromosome 11q23. [345] Because the genetic defects responsible for most of these disorders (*RET*, *VHL*, *NF-1*) have been identified, genetic testing is available for patients with a positive family history and for patients with apparently sporadic disease.[26,174,347,348 and 349]

Thus far, mutations in exons 10, 11, and 14 of the RET proto-oncogene (located on chromosome 10q11.2) lead to pheochromocytomas in patients with MEN IIA, and mutations in exon 16 of *RET* are found in more than 95% of patients with MEN IIB.[26,350,351] Somatic *RET* mutations are detected in fewer than 20% of sporadic pheochromocytomas.[352,353] In patients with VHL disease, mutations of the VHL tumor suppressor gene on chromosome 3p25.5 predispose to the development of pheochromocytomas (VHL type 2).[343,348] Similar genotype-phenotype correlations are not available for NF-1, because (a) pheochromocytomas in NF-1 occur in fewer than 2% of patients, (b) NF-1 has pathognomonic clinical features, and (c) the NF-1 gene is large and mutation analysis is cumbersome. Neurofibromatosis type 2 is not associated with pheochromocytoma.

### Pathology and Patterns of Spread

Pheochromocytomas and paragangliomas can occur in all locations where chromaffin tissue is found. [334,337] Approximately 85% of these tumors are located in the adrenal glands. Common extraadrenal sites are sympathetic ganglia near the kidney and the organ of Zuckerkandl. Pheochromocytomas may occur multifocally and bilaterally, especially in hereditary tumor syndromes. Approximately one-third of affected children have multiple tumors. [338,341]

Most pheochromocytomas measure less than 5 cm. They are vascular tumors and commonly contain cystic, necrotic, or hemorrhagic areas. Tumor cells contain typical catecholamine storage granules. MEN II–associated pheochromocytomas are surrounded by extratumoral adrenomedullary hyperplasia, in contrast to VHL-associated pheochromocytomas. Intracytoplasmic hyaline globules are found commonly in MEN II pheochromocytomas, whereas tumor cells in VHL pheochromocytomas demonstrate a clear-cell pattern. On histologic grounds, the diagnosis of malignant pheochromocytoma cannot be made. Malignancy is defined only by the presence of clinical, distant metastases. Bone, liver, lung, and lymph nodes are the most common sites for metastases from a pheochromocytoma. [354]

### Endocrine Manifestations

Sustained or paroxysmal arterial hypertension is the most common sign and is found in 80% of pediatric patients. [338,341,367] In contrast to adult patients with pheochromocytoma, children with this tumor experience sustained hypertension much more commonly than paroxysmal attacks. Sustained hypertension in children due to pheochromocytoma usually is refractory to conventional antihypertensive treatment. Other symptoms and signs of catecholamine excess include headache, sweating, palpitations, flushing, anxiety, tremor, nausea and vomiting, abdominal or chest pain, and visual disturbances. Fatigue and exhaustion may follow a paroxysmal attack. Weight loss, constipation, and low-grade fever are less commonly present. Polyuria and polydipsia may occur as a result of hyperglycemia, which is found in approximately one-third of pediatric pheochromocytoma patients. Orthostatic hypotension, which is seen mainly in patients with MEN II pheochromocytoma, may occur as a result of inadequately functioning neurovascular reflexes or predominant release of epinephrine from the tumor. Pheochromocytomas rarely cosecrete other hormones such as vasoactive intestinal peptide (VIP) and ACTH. In these cases, additional symptoms attributable to the respective hormone secretion (i.e., watery diarrhea, hypokalemia in the case of VIP; Cushing's syndrome in the case of ACTH) can be present in the pediatric pheochromocytoma patient. Physical examination is usually normal but can reveal hypertension and its consequences of increased heart size and retinopathy. If the patient does not have NF-1 or MEN IIB, both of which have typical pathognomonic clinical features (i.e., café au lait spots, neurofibromas, and the like in NF-1; mucosal neuromas with blubbery lips, marfanoid habitus, and the like in MEN IIB), the physical examination may be unremarkable, as no specific signs or physical findings are associated with pheochromocytoma.

### Diagnosis

Currently available assays for catecholamines and their metabolites have simplified the diagnosis of pheochromocytoma ( Fig. 37-5). Plasma and urinary catecholamines, including free metanephrine and normetanephrine, are usually sufficient to confirm the diagnosis. Some malignant tumors may secrete large amounts of dopamine, leading to increased plasma concentrations and urinary excretion of the dopamine metabolite homovanillic acid. Dopamine is also the principal active

catecholamine produced by ganglioneuromas and neuroblastomas, leading to selectively elevated urinary homovanillic acid levels. During blood sampling and urine collections, drugs (e.g., acetaminophen) and foods that stimulate catecholamine secretion or interfere with the catecholamine assays must be eliminated for a certain amount of time (e.g., acetaminophen for at least 5 days).[336,355]

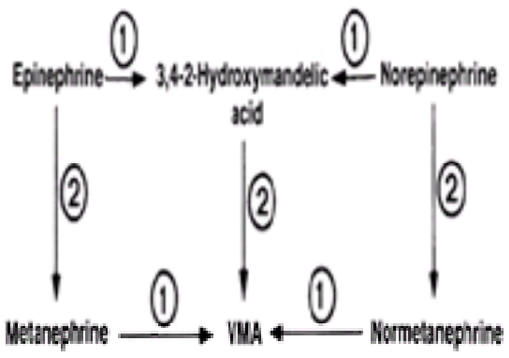



**FIGURE 37-5.** Catecholamine metabolism pathway. Numbers in circles represent metabolizing enzymes: 1, monoamine oxidase; 2, catechol-O-methyltransferase. VMA, vanillylmandelic acid.

In patients with brief and infrequent paroxysms separated by symptom-free intervals, confirmation of the diagnosis may be difficult, if such a diagnosis is based on the measurement of plasma catecholamines. Preferentially, the measurement of plasma metanephrine concentrations should be undertaken, as production of metanephrines by tumor cells occurs continuously and independently of catecholamine release. If plasma metanephrine concentrations are less than threefold elevated, performance of the glucagon test should be considered to confirm further the biochemical diagnosis of pheochromocytoma. In such cases, it may be useful to induce a paroxysm (Table 37-3).[357,358] This test should be done under the supervision of an endocrinologist, having phentolamine and a b-adrenergic blocker (in case of an epinephrine-secreting tumor) at the bedside. Injection of 1 mg glucagon intravenously can induce an attack in most patients with pheochromocytoma. The crucial time point for plasma catecholamine collection and subsequent measurements is 2 minutes after the injection of glucagon.

Another confirmatory test is the clonidine suppression test.[359] Clonidine (0.3 mg per 70 kg), an $a_2$-adrenergic agonist that suppresses the central sympathetic system, usually fails to decrease catecholamine secretion in patients with pheochromocytoma. However, the specificity of this test is not higher than 80%.

If the chemical tests are inconclusive, periodical following of a patient or initiation of a therapeutic trial with phenoxybenzamine hydrochloride may be useful. After the diagnosis of pheochromocytoma has been established, the tumor must be localized to facilitate its surgical removal. CT or MRI scans frequently are sufficient. A scintigraphic procedure in which $^{123}$I- or $^{131}$I-labeled meta-iodobenzylguanidine (MIBG) is injected leads to detectable images of pheochromocytomas 24 to 72 hours later.[360,361] The procedure is highly specific and can detect pheochromocytomas not detected by CT. However, not all pheochromocytomas produce detectable images, and so other imaging modalities—including octreoscan or positron emission tomography scanning—should be considered. Catecholamine measurements in blood samples obtained by percutaneous venous catheterization at various points along the inferior vena cava or renal, adrenal, and jugular veins can be of great value in locating small tumors.

### Treatment

As soon as the diagnosis has been confirmed, therapy with adrenergic antagonists should be initiated.[5,346,362,363 and 364] This treatment allows reduction of symptoms, lowering of blood pressure, amelioration of paroxysms, and expansion of the vascular bed and blood volume. A few days are required for preoperative preparation of patients. The agents used are primarily phenoxybenzamine (Dibenzyline), a noncompetitive a $_1$-adrenergic antagonist with a long effect (half-life of 36 hours); a calcium channel blocker; and metyrosine (Demser). Postural hypotension may be seen at the beginning of therapy with these agents. Occasionally, small doses of the b-blocking agent propranolol is required to control tachycardia or arrhythmias. Medical preparation of the patient decreases the risks of anesthesia and surgery.[5,29,365,366,367,368 and 369]

Abdominal tumors are approached transabdominally to allow exploration of the adrenal glands and the abdominal sympathetic ganglia. If bilateral adrenal tumors are found, both adrenal glands are removed, and glucocorticoid and mineralocorticoid replacement is required. The prognosis after successful surgery is excellent, as fewer than 10% of intraadrenal pheochromocytomas are malignant.

Patients with unresectable malignant tumors or metastases can be managed medically for some time. Phenoxybenzamine or a-methyltyrosine can be employed. Long-term survival of these patients has been reported but is rare. Usually, life expectancy with a malignant pheochromocytoma is less than 5 years. At present, treatment modalities include $^{131}$I-MIBG, chemotherapy, octreotide, and tumor chemoembolization.[370,371,372,373 and 374] On occasion, bone metastases respond well to irradiation given for symptomatic relief. Chemotherapy or radiotherapy alone or in combination have been disappointing in patients with unresectable malignant pheochromocytomas.

## TUMORS OF THE GASTROENTEROPANCREATIC UNIT

The rare tumors that arise from endocrine cells of gastroenteropancreatic origin and secrete peptide hormones have collectively been called *APUDomas*. The term has prevailed because these tumors apparently arise from neuroectodermal cells that have the ability to take up, decarboxylate, and store aromatic amine precursors, referred to as *amine precursor uptake and decarboxylation*, or *APUD*.[4,5 and 6,375,376,377 and 378] However, this concept is outdated, as a large body of evidence now exists to prove that essentially every epithelial stem cell can differentiate to a neuroendocrine tumor cell.[15,16,378] Most so-called APUDomas are found in the pancreas, but a few occur in the wall of the GI tract or in the retroperitoneum. Most of these tumors probably arise from pluripotential stem cells (i.e., nesidioblasts) in pancreatic ducts. Although many APUDomas contain more than one type of endocrine cell and secrete more than one hormone, they usually are named after the hormone most responsible for the clinical manifestations.

Diagnosis is made by measuring an elevated level of a gastroenteropancreatic hormone in the blood. Measurements are made on basal samples or after provocation (Table 37-3). Finding APUDomas usually is difficult during laparotomy. Preoperative localization by CT, MRI, pancreatic arteriography, octreotide scintigraphy, or percutaneous transhepatic venous sampling of portal vein tributaries for measurement of the suspected hormones frequently is attempted.[4,5 and 6,376,377,379,380 and 381]

Approximately 50% of APUDomas are malignant at the time of diagnosis. APUDomas are rare in childhood. The most frequent type is the insulinoma. Gastrinoma, VIPoma, glucagonoma, and somatostatinoma occur less frequently. The overall incidence of concurrent endocrine tumors with APUDomas is high; approximately 10% to 20% of patients with gastrinomas and 5% of patients with insulinoma have evidence of MEN I. Neuroendocrine tumors of the pancreas also are seen in VHL disease. No specific genetic defects have been associated with APUDomas. Amplification of the HER-2/neu proto-oncogene, a member of the ERBB-like oncogene family, was demonstrated in endocrine tumors of the GI tract.[382]

Treatment of these tumors should be individualized. The indolent nature of many APUDomas means that a period of watchful waiting is appropriate for certain patients. Conversely, aggressive surgical resection is recommended for metastatic tumors. Patients with the MEN syndromes may be required to have more extensive surgery because the pancreatic islet cell tumors associated with this condition are small and multifocal and their diagnosis is difficult preoperatively. Patients with Zollinger-Ellison syndrome in the scope of MEN I have a poor prognosis, and surgery for this condition is not curative, as opposed to surgery in patients with sporadic gastrinomas, which can be curative.[381] Systemic therapy for APUDomas may be separated into that directed against the cancerous cells and that which provides supportive care. Octreotide, a long-acting analog of the endogenous somatostatin, is useful in the treatment of insulinomas, nesidioblastosis, VIPomas, and the diarrhea associated with the latter (i.e., Verner-Morrison syndrome). Chemotherapeutic agents used for these tumors include fluorouracil, doxorubicin, streptozotocin, dacarbazine, etoposide, and cisplatin. The combination of streptozotocin plus doxorubicin is superior to that of streptozotocin plus fluorouracil, resulting in a significant improvement in overall survival for patients with pancreatic islet cell tumors. The latter combination appears to be more beneficial for patients with other

carcinoid tumors. Interferon therapy has been used for treating neuroendocrine tumors of the GI tract in adults. [378,383] In addition to surgical resection, several liver-directed therapies, such as arterial chemotherapy, hepatic arterial occlusion, and hepatic arterial embolization and chemoembolization, can be directed against liver metastases of these tumors.

### Insulinoma

Insulin-secreting tumors of the islets of Langerhans are called *insulinomas*.[384,385] Most of these tumors are single and benign, although a small percentage are multiple, and a small percentage are malignant. Most insulinomas are located in the pancreas. Diffuse pancreatic b-cell hyperplasia or nesidioblastosis can also be associated with excess insulin secretion and hypoglycemia in children. [383,386,387,388,389,390,391 and 392]

The signs and symptoms are predominantly those of subacute glycopenia, primarily recurrent central nervous system dysfunction at times of physical exertion or fasting. Acute hypoglycemic episodes with adrenergic discharge symptoms (e.g., sweating, hunger, tremor, seizures) can occur. Frequently, the patients are obese because of the lipogenic and antilipolytic effects of insulin. [384,386,387,393]

Pancreatic b-cell tumors do not reduce their secretion of insulin in the presence of hypoglycemia. A serum insulin level of 10 mU per mL or more with concurrent plasma glucose concentrations of less than 40 mg per dL suggests hyperinsulinism. Fasting of the patient with frequent sampling for plasma insulin and glucose concentrations is the best available test and provides a diagnosis for most patients. Alternatively, hypoglycemia caused by exogenous insulin (0.1 unit per kg per hour given intravenously) fails to cause suppression of plasma C peptide (a marker of endogenous insulin secretion) to less than 50% of the baseline value in patients with insulinomas.

The treatment of choice for insulin-secreting tumors is surgical resection. Preoperatively or occasionally postoperatively, patients are treated with oral diazoxide (5 to 15 mg per kg per day in divided doses).[384,386,393] Side effects include sodium retention (which can be treated with concomitant thiazide administration), gastric irritation, and generalized hypertrichosis. If the surgeon cannot locate the tumor, a blind pancreatectomy of the distal two-thirds of that organ can be performed, although the success rate is low.[389] Streptozotocin alone or in combination with doxorubicin has proved beneficial in adult patients with carcinomas of the islet cells. [394,395 and 396] Benign tumors either respond poorly or do not respond.

### Gastrinoma

Gastrinomas are gastrin-secreting tumors that cause gastric acid hypersecretion and the Zollinger-Ellison syndrome. [4,5,380,381,397,398,399 and 400] Most gastrinomas occur in the pancreas, but others are found in the duodenum or, rarely, in the antrum. Gastrinomas usually are small and, frequently, locating them is difficult, even during laparotomy. These tumors are identified as malignant if metastases or blood vessel invasion are found. The histologic pattern is similar for malignant and benign tumors.

The symptoms of gastrinomas are manifestations of peptic ulcer disease and its complications. Some patients present with diarrhea due to passage of large amounts of acid into the duodenum.

Hypergastrinemia in the presence of acid hypersecretion is pathognomonic of gastrinoma. [4,5,379,381,398,401,402 and 403] Plasma gastrin levels usually exceed 500 pg per mL (normal being less than 200 pg per mL). Patients with borderline hypergastrinemia (200 to 500 pg per mL) should undergo the secretin stimulation test (2 units per kg by intravenous bolus); a rise in plasma gastrin to more than 1,500 pg per mL within 15 minutes is diagnostic. An upper GI series usually shows ulceration of the duodenal bulb, prominent gastric rugal folds, and edema of the small bowel mucosa. Selective angiography, CT scanning, or octreotide scintigraphy can localize the pancreatic tumor.

Patients with gastrinomas should be started on $H_2$-blocking agents such as cimetidine or ranitidine. [4,5,381,404,405 and 406] This therapy is beneficial initially but becomes less effective with time. Tumor resection is ideal but is feasible in only a small percentage of patients. Laparotomy and removal of solitary tumors simultaneously with vagotomy may provide cure or enhance the effectiveness of $H_2$-blocking agents.

Total gastrectomy remains the treatment of choice for patients with complications of ulcer disease and for those whose disease is not controlled satisfactorily by the $H_2$-blockers. These patients require lifelong therapy with iron and vitamin $B_{12}$ replacement.

The prognosis for patients with sporadic gastrinomas is generally good, and most patients lead a relatively normal life after gastrectomy. Most patients with malignant gastrinomas live for many years. A few patients who have aggressive, rapidly growing, and metastasizing tumors may respond to streptozotocin with or without fluorouracil.[407]

### Vasoactive Intestinal Peptide–Secreting Tumor

VIPomas are pancreatic tumors that secrete VIP and are associated with a syndrome of watery diarrhea, hypokalemia, and achlorhydria (i.e., pancreatic cholera).[4,5,408,409,410,411,412,413,414,415 and 416] In addition to VIP, serotonin, substance P, calcitonin, pancreatic polypeptide, and some of the prostaglandins may be present in high concentrations in the blood.

Complete removal of the tumor is curative. On occasion, the tumor cannot be found, and subtotal pancreatectomy is required. Malignant VIPomas may respond to the combinations of chemotherapeutic agents mentioned in the previous section. [417]

### Glucagonoma

Glucagonomas are pancreatic tumors that secrete glucagon. [4,5 and 6,418,419,420 and 421] The syndrome produced is characterized by migratory necrolytic dermatitis, weight loss, stomatitis, anemia, and hyperglycemia or frank diabetes mellitus. Glucagonomas can be benign and confined to the pancreas or malignant with metastases to the liver, regional lymph nodes, adrenal glands, or bones. Surgical removal, if feasible, is indicated.

### Somatostatinoma

Somatostatinomas are pancreatic tumors that secrete somatostatin.[4,5 and 6,422,423 and 424] The syndrome produced is characterized by hyperglycemia or frank diabetes mellitus, diarrhea, and malabsorption. Most somatostatinomas are malignant and give rise to hepatic metastases. Surgery is indicated if the disease is localized. Chemotherapy with streptozotocin may be helpful.

## ECTOPIC HORMONE–SECRETING TUMORS

Ectopically secreted hormones are peptides that cause endocrine syndromes similar to those caused by the entopically produced or exogenously administered “parent” hormones (Table 37-1).[4,5,6 and 7,425,426 and 427] Frequently, however, ectopic hormones are biochemically different from the parent hormones. For example, tumors may manufacture large precursors that possess a fraction of the biologic activity of the parent hormone. [20] The ectopic hormone cannot always be confirmed as a moiety similar to the true hormone. If the chemical identity of the hormone cannot be confirmed, the hormones are given the name of the normal circulating hormone and the suffix *-like*.

Many of the ectopic hormone–secreting tumors have been described only in adults, but it is theoretically possible that they can develop also in children. Ectopic CRH-secreting tumors (e.g., metastatic prostatic carcinoma, ganglioneuroma, lung carcinoma) are rare and have been described only in adults. [38,428,429 and 430] These tumors are associated with Cushing's syndrome. Ectopic ACTH-secreting tumors have been described in adults and children. [6,431,432,433 and 434] They are associated with severe Cushing's syndrome and are produced by carcinomas of the lung, thymus, pancreas, thyroid, adrenal medulla, and other tissues. Ectopic tumors secreting GH-releasing hormone (GHRH) have been described in adults and adolescents and are associated with gigantism and acromegaly. [37,435,436,437,438,439,440,441,442 and 443] A

primary jejunal tumor with lymph node and liver metastases and a metastatic foregut carcinoid tumor, both of which secrete GHRH, have been reported. No ectopic tumors secreting GH have been described in children. In adults, ectopic GH synthesis has been attributed to pancreatic, gastric, bronchial, and mammary carcinomas.[6,444,445,446 and 447] Ectopic chorionic gonadotropin secretion has been described in children, causing precocious puberty in boys by stimulating Leydig cells. [6] Chorionic gonadotropin is secreted by placental trophoblastic neoplasms, testicular and pineal tumors, hepatoblastomas, and carcinomas of the lung, stomach, pancreatic islet cells, and colon.[448,449,450,451,452,453 and 454] Tumors secreting the inactive alpha subunit, which then serves as a marker, include malignant insulinomas, gastrinomas, VIPomas, and intestinal or pulmonary carcinoids.[449,455]

Ectopic AVP-secreting tumors producing the inappropriate antidiuretic hormone syndrome have been reported in adults. [6,456] Several neoplasms, commonly small-cell or oat-cell carcinomas of the lung and carcinoma of the colon or, less commonly, prostatic or adrenocortical carcinomas, have been associated with ectopic AVP secretion.

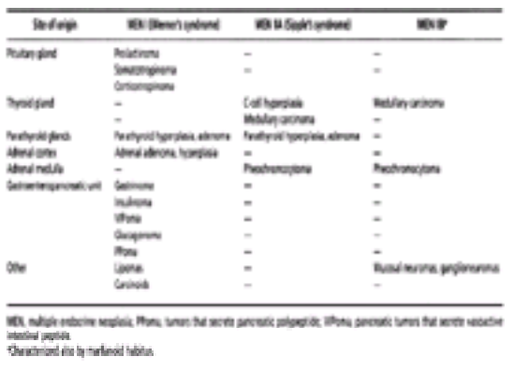
Ectopic calcitonin secretion has been observed with bronchial carcinoids and with lung, breast, and other tumors. [456] This hormone does not produce an endocrine syndrome. Ectopic PTH or PTH-like substances have been described with many cancers.[457,458,459 and 460] Ectopic PTH is presumably a form of a PTH precursor that remains unrecognized by many antibodies raised against mature PTH. Osteoclast-activating factor or some prostanoic substance stimulating bone resorption may be the PTH-like hormone.

No ectopic steroid or thyroid hormone syndrome exists, because it would entail the random synchronous activation of multiple enzymes required for biosynthesis of these hormones. However, adrenal rest tumors can be found in many ectopic areas, primarily the liver, pelvis, or testes, which can “ectopically” secrete such steroid hormones as cortisol.

Many hormones of the gastroenteropancreatic unit are secreted ectopically by several tumors. Some nonpancreatic tumors have been associated with hypoglycemia.[20] These tumors are fairly large by the time hypoglycemia is noticed and include retroperitoneal fibromas and fibrosarcomas, some hepatomas, some tumors of the adrenal cortex, and others. This phenomenon, known as *tumor hypoglycemia*, is caused by “big IGF-2,” which has cross-reactivity with the insulin receptor. Most affected patients are in the final stages of a neoplastic disease. Treatment includes surgical extirpation or irradiation of the tumor and frequent feedings, intravenous glucose infusions, or administration of glucocorticoids. Ectopic secretion of gastrin, glucagon, somatostatin, and VIP has been described.[4,6,461,462,463 and 464]

## MULTIPLE ENDOCRINE NEOPLASIA SYNDROMES

MEN syndromes are familial disorders in which neoplastic changes arise simultaneously in more than one endocrine gland. [3,4,5,6 and 7,26,174,175,235,465,466] The neoplastic changes include hyperplasia, benign adenomas, and carcinomas. Three distinct patterns of glandular involvement and overlapping or atypical combinations of gland involvement have been described. The three combinations are referred to as *MEN I*, *MEN IIA*, and *MEN IIB* (the latter sometimes still being labeled as *MEN III*; Table 37-5).[222,239,465,467,468,469,470,471 and 472,474] Carney complex is a distinct type of MEN that is characterized by the additional features of recurrent cardiac and skin myxomas and lentigines. Other genetic disorders with multiple endocrine tumors are VHL disease (i.e., pheochromocytoma, neuroendocrine tumors of the pancreas), NF-1 (pheochromocytoma), and the Li-Fraumeni syndrome (adrenocortical carcinoma).



| Site of origin | MEN I (Wermer's syndrome) | MEN IIA (Sipple's syndrome) | MEN IIB |
|---|---|---|---|
| Pituitary gland | Prolactinoma | – | – |
| | Somatotropinoma | – | – |
| | Corticotropinoma | | |
| Thyroid gland | – | C cell hyperplasia | Medullary carcinoma |
| | – | Medullary carcinoma | – |
| Parathyroid glands | Parathyroid hyperplasia, adenoma | Parathyroid hyperplasia, adenoma | – |
| Adrenal cortex | Adrenal adenoma, hyperplasia | – | – |
| Adrenal medulla | – | Pheochromocytoma | Pheochromocytoma |
| Gastroenteropancreatic unit | Gastrinoma | – | – |
| | Insulinoma | – | – |
| | VIPoma | – | – |
| | Glucagonoma | – | – |
| | PPoma | – | – |
| Other | Lipoma | – | Mucosal neuroma, ganglioneuroma |
| | Carcinoid | – | – |

MEN, multiple endocrine neoplasia; PPoma, tumors that secrete pancreatic polypeptide; VIPoma, pancreatic tumors that secrete vasoactive intestinal peptide.
*Characterized also by marfanoid habitus.

**TABLE 37-5. COMPARISON OF CLUSTERS OF INVOLVED TUMORS IN MULTIPLE ENDOCRINE NEOPLASIA SYNDROMES**

### Epidemiology and Genetics

Approximately one-half of the MEN and Carney complex cases occur sporadically and represent de novo (somatic) mutations of the responsible genes. The remaining patients belong to kindreds, in which these conditions are inherited in an autosomal dominant manner with variable penetrance and expression. Affected relatives with the same type of MEN syndrome may have different neoplasms that appear at different ages.[26,476] Combinations of multiple endocrine tumors that do not fit any of the distinct MEN syndromes may occur in a single patient.[477]

The gene for MEN I has been localized to chromosome 11q13 and subsequently identified. It acts as a tumor suppressor gene and encodes the protein menin. Menin's function still remains unknown.[235,478] Germline mutations of the RET proto-oncogene on chromosome 10q11.2 have been described in almost all patients with MEN IIA and MEN IIB. *RET* comprises 21 exons, with 6 so-called hotspot exons (numbers 10, 11, and 13–16). Patients with MEN IIA have mostly germline mutations in exons 10 and 11, whereas 95% of patients with MEN IIB have germline mutations in exon 16 at codon 918. Transfection assays with *RET* have shown that its mutations lead to a “gain of function”—that is, are activating mutations. The encoded protein of *RET* is a receptor tyrosine kinase that becomes constitutively activated when mutations in *RET* are present. The mechanisms by which *RET* mutations lead to tumor formation *in vivc* remain largely unknown but have recently been elucidated. It may well be that the mutated *RET* allele exerts a dominant effect if imbalanced with the wild-type allele. [350] Interestingly, *RET* mutations can also be found in patients with Hirschsprung's disease, a disorder in which part of the colon is aganglionic, leading to a megacolon formation. This may be explained by the expression pattern of *RET*: The three tissues principally involved in MEN II are thyroid C cells, adrenal medulla, and intestinal autonomic ganglia, all derived from neural crest cells.[26,176,347,466] The genetic locus for Carney complex, an autosomal dominant heterogeneous disorder with variable expression and evidence of genetic anticipation, was mapped initially to chromosome 2 (2p16) and recently was reassigned to chromosome 17q24. [172,301,309]

The VHL gene acts as a tumor suppressor gene, which, according to Knudson's hypothesis, requires two hits to cause tumorigenesis: The first hit is an inactivated copy of one *VHL* allele by a germline mutation, deletion, or hypermethylation, and the second hit is an inactivation of the second VHL gene copy by point mutation, gene deletion, or hypermethylation.

#### Multiple Endocrine Neoplasia Type I

The parathyroids are the glands most frequently affected in MEN I (i.e., Werner's syndrome). [236] Hyperparathyroidism, usually due to hyperplasia of all four glands, occurs in approximately 90% of patients. Islet cell tumors occur in approximately 20% of patients. Nearly 50% of GI endocrine tumors in MEN I secrete gastrin. VIPomas, glucagonomas, somatostatinomas, and tumors secreting pancreatic polypeptide (PPomas) have also been reported. More than one-half of the gastrinomas, VIPomas, glucagonomas, and PPomas associated with MEN I are malignant, whereas most of the insulinomas are benign.[236,476]

Pituitary involvement in MEN I usually occurs as a solitary adenoma. Signs and symptoms of a pituitary adenoma are apparent in approximately 20% of MEN I patients. The most common adenomas are prolactinomas, and the second most common are somatotropinomas. Corticotropinomas are third most common but are rare (approximately 5%). Chromophobe adenomas can also occur (5%). Adrenocortical involvement includes silent adenomas, adrenocortical hyperplasia, cortisol-secreting adenomas and, rarely, carcinomas. Benign and malignant thymic and bronchial carcinoid tumors can be associated with MEN I. Single or multiple lipomas and angiofibromas are observed in approximately 30% of MEN I patients.[27,479] The clinical picture of the endocrine tumors that compose the MEN I syndrome

is generally the same as that for sporadic tumors. Hyperparathyroidism, the Zollinger-Ellison syndrome, hypoglycemia, pancreatic cholera, migratory necrolytic erythema, specific and nonspecific symptoms from pituitary adenomas, Cushing's syndrome from cortisol-secreting adrenal adenomas or ectopic ACTH syndrome from gastrinomas or carcinoids, carcinoid syndrome, and gigantism or acromegaly can occur.

Laboratory findings are related to the specific hormonal syndrome. Therapy is directed toward the specific tumor and endocrine syndromes and is referred to in other parts of this chapter.

### Multiple Endocrine Neoplasia Type IIA

The thyroid C cells are most frequently involved in patients with MEN IIA (i.e., Sipple's syndrome), with medullary carcinoma of the thyroid occurring in approximately 95% of the patients.[26,175,465,469] Pheochromocytoma occurs in approximately one-half of patients, although probably all patients with a germline *RET* mutation have adrenomedullary hyperplasia from birth.[480] These tumors usually are intra-adrenal and frequently bilateral and multifocal. Approximately one-fifth of patients develop frank hyperparathyroidism, mostly associated with parathyroid adenoma hyperplasia.

The clinical and laboratory findings of MEN IIA are those expected on the basis of the hormones secreted by the tumors (catecholamines, PTH). Approximately 50% of patients with MEN IIA and pheochromocytoma are asymptomatic despite having continuous metanephrine secretion into the bloodstream from the adrenomedullary tumor. Treatment is directed to the type of tumor (total thyroidectomy for medullary thyroid cancer, adrenalectomy for pheochromocytoma, parathyroidectomy for hyperparathyroidism).

### Multiple Endocrine Neoplasia Type IIB

All patients with MEN IIB have some aspect of a distinct marfanoid phenotype.[1,26,174,175,470,473,476] This is characterized by a slender body build, long and thin extremities, abnormal laxity of joints and, in many cases, a high arched palate, pectus excavatum, or pes cavus. The facies is characterized by enlarged, thick (blubbery) lips, a result of embedded mucosal neuromas. Mucosal neuromas also are observed on the surface of the lips and tongue and may be found on the eyelids and the cornea. Ganglioneuromas may be present diffusely at any level in the GI tract, causing constipation or diarrhea due to abnormal control of intestinal motility.

Medullary thyroid carcinoma is particularly aggressive in MEN IIB and occurs frequently in childhood cases (mean patient age, 20 years), 10 to 15 years earlier than the mean age of presentation of MEN IIA. Pheochromocytomas behave similarly to those observed in MEN IIA.

Treatment of MEN IIB includes standard therapy for medullary thyroid carcinoma and pheochromocytoma. However, because medullary thyroid carcinoma in this MEN type is very aggressive, total thyroidectomy may become necessary as early as the first year of life.[175,475] Superficial mucosal neuromas may be removed if they cause a cosmetic problem. Constipation or diarrhea should be treated symptomatically. As many as 30% of patients require laparotomy and colon segment excision for megacolon.

### Carney Complex

Carney complex is a familial multiple neoplasia and lentiginosis syndrome.[481] Historically, the complex was characterized by the association of PPNAD, a pituitary-independent, primary adrenal form of hypercortisolism; lentigines, ephelides, and blue nevi of the skin and mucosae; and a variety of nonendocrine and endocrine tumors. The latter include myxomas of the skin, heart, breast, and other sites; psammomatous melanotic schwannoma; GH-producing pituitary adenoma; testicular Sertoli cell tumor; and possibly other benign and malignant neoplasms, including tumors of the thyroid gland and ductal adenoma of the breast. The existence of the complex as an unrecognized, inherited syndrome was first suggested in 1982 by Schweizer-Cagianut et al.,[309] but patients with lentigines (lentigo simplex) or ephelides and blue nevi had been described earlier under different pseudonyms, such as *NAME* (for *n*evi, *a*trial myxoma, *m*yxoid neurofibromas, and *e*phelides) and *LAMB* (for *l*entigines, *a*trial myxoma, *m*ucocutaneous myxoma, and *b*lue nevi) syndromes. Carney complex shares with the other MENs the presence of endocrine tumors and the characteristic spotty skin pigmentation, in addition to the familial lentiginoses, among which are the Peutz-Jeghers syndrome, the syndrome of lentigines and hypertrophic cardiomyopathy (known by the acronym *LEOPARD*), and the syndrome of arterial dissections with lentiginosis.[28,172,301,481] The tumors of the complex can appear as early as in the first decade of life. Cardiac myxomas can cause sudden death, and so echocardiographic screening for the presence of these tumors in all affected family members is essential. Open heart surgery is always recommended for the excision of these tumors, which can cause embolization or myocardial infarction if left unresected. PPNAD can be an indolent cause of mild, periodic Cushing's syndrome that may not be clinically detectable.[482]

### Prognosis

The prognosis for patients with MEN I generally is good in the presence of a discrete parathyroid, pancreatic islet, or pituitary adenoma. Parathyroid hyperplasia occurs in approximately one-third of patients. Pancreatic islet cell carcinoma and carcinoids are slowly progressive.

The prognosis for patients with MEN IIA generally is good also. The risk of postoperative recurrence of medullary thyroid carcinoma and pheochromocytoma is diminished if the tumor is excised early, before the disease becomes extensive. Thus, total thyroidectomy is recommended for children older than 5 years who have a family history of MEN II and a *RET* germline mutation, as they will develop medullary thyroid cancer on the basis of thyroid C cell hyperplasia.[26,175,475,483] Hyperparathyroidism in this syndrome has a good prognosis.

The prognosis for patients with MEN IIB is worse than that for MEN IIA patients. Medullary carcinomas in MEN IIB patients generally are more aggressive, and the patients have a 50% 10-year survival rate.

Patients with Carney complex generally have a good prognosis. However, frequent recurrences of the cardiac and skin myxomas and the other tumors are a significant cause of morbidity and mortality in these patients. In a series of 51 patients, sudden death or a near-death event occurred in 16%, all during childhood, adolescence, and young adulthood.[172]

Screening is of paramount importance in all forms of MEN and Carney complex, because earlier or even preventive therapy improves the prognosis remarkably. Patients at risk include those with known MEN, a positive family history of MEN, ganglioneuromas and cutaneous neuromas, a marfanoid somatic phenotype, Zollinger-Ellison syndrome, parathyroid hyperplasia, multicentric medullary carcinoma of the thyroid, or multicentric or bilateral pheochromocytomas. Screening tests include measurements of serum calcium, gastrin, glucose, and prolactin levels in MEN I; basal and stimulated (calcium gluconate or pentagastrin, omeprazole) plasma calcitonin, 24-hour urinary free catecholamines, metanephrines, and vanillylmandelic acid, and serum calcium levels in MEN IIB; and basal and stimulated plasma calcitonin and 24-hour urinary free catecholamines, metanephrines, and vanillylmandelic acid levels in MEN IIA ( Table 37-3). Patients with Carney complex should be screened frequently by echocardiography for cardiac myxomas and clinically and biochemically for PPNAD, somatotropinoma, and other tumors.[107,191,481,484,485,486] and [487]

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# 38

# MANAGEMENT OF INFREQUENT CANCERS OF CHILDHOOD

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## INTRODUCTION

The preceding chapters have dealt with the principles of pediatric oncology and the management of more common tumors of childhood. This chapter describes some of the less frequently encountered malignant tumors. Much of the information presented may be related to tumors of adults rather than children, but the basic principles of treatment remain the same. Pediatric hematologists-oncologists require some knowledge of the diagnosis, management, and treatment derived from the more extensive experience of medical, surgical, and radiation oncologists in dealing with these rare tumors of childhood.

Some information regarding the staging of these tumors is required by the pediatric oncologist, who should confer with surgeons and pathologists to give information to patients, parents, and radiation oncologists. References to the fifth or subsequent edition of the American Joint Committee on Cancer (AJCC) Staging Handbook [1] is required for such tumors as gastric, colon, renal cell, pancreatic, and nasopharyngeal carcinomas, as well as melanoma.

The tumors are discussed in descending anatomic order, from the nasopharynx through the trunk, to the skin. Some references that are pertinent to adult tumors as well as childhood tumors are included.

## OROPHARYNGEAL CANCER

Oropharyngeal tumors are rare yet challenging to pediatric oncologists, surgeons, and radiotherapists. The most common of these cancers is nasopharyngeal

carcinoma, which is discussed separately.

There is increasing evidence of the use of smokeless tobacco products by adolescent and preadolescent boys. [2,3,4 and 5] Known carcinogenic agents in smokeless tobacco may be associated with oral carcinoma, principally squamous cell carcinoma. [6,7,8,9,10 and 11] Although rare at the present time, this may become a significant problem for the future generations if there is continued use of these products. Risk is increased with increasing length of exposure, with greatest risk for anatomic sites where the smokeless tobacco is held in contact for the longest time. Figures related to incidence of head and neck carcinomas in patients younger than 20 years are not available except for nasopharyngeal, parotid, and minor salivary gland carcinomas. [12]

It has been noted that 8% to 36% of male high school and college age students regularly use smokeless tobacco products. The mean age for initiating the use of these products is 12 years. The average use of chewing tobacco or snuff has been estimated at six times daily. The average duration per dip or chew is 1 hour. [6,12]

There is concern that smokeless tobacco products may lead to cardiovascular disease as well as increased risk of oral cancer, primarily because of the pharmacological properties of nicotine and other constituents in tobacco smoke. There is, however, no evidence to date of relationships between smokeless tobacco use and bladder cancer; yet there have been suggestions of links between smokeless tobacco and prostate cancer.

The National Youth Tobacco Survey of more than 15,000 students showed the current cigarette smoking habits among middle and high school students by race and ethnicity.[13] This study indicated that although middle school smoking rates were similar for blacks, Hispanics, and whites, black high school students had a significantly lower rate of cigarette smoking than did the other two groups (15.8% for black vs. 32.8% for white and 25.8% for Hispanic). [10]

The physical changes associated with chewing tobacco or using snuff include alterations in the texture, color, and contour of the mucosal lining and periodontal degeneration.[4] This has been seen in more than half of the teenagers surveyed for changes related to smokeless tobacco. Leukoplakia, a precancerous condition, occurs in more than one-third of the users. Other lesions may take on the characteristics of ulcers, blisters, and gum and lip lesions, and include lesions induced by cancer chemotherapy agents.[14]

Pediatricians and health departments should continue to assist in educating middle school and high school students regarding the use of these products. Without specific changes in the use of these products, there most probably will be an inordinate number of oral cancers of young adults within the next decades.

Benign tumors and other cancers also may involve the oropharynx or neck. [15,16,17,18,19,20,21,22,23 and 24] These include dermoid cysts, leiomyosarcomas, myofibromas, fibromatoses, cystic hygromas, hemangiomas, and teratomas. Life-threatening obstructive complications requiring surgical intervention may be required but would rarely require the use of chemotherapy or radiation therapy.

## NASOPHARYNGEAL CARCINOMA

Nasopharyngeal carcinoma has been known in the past as lymphoepithelioma or epidermoid carcinoma. [25,26] It is a primary malignancy of the nasopharyngeal epithelium that accounts for one-third of the nasopharyngeal neoplasms in children. About one-third of these neoplasms of the undifferentiated type are diagnosed in adolescents or young adults. [27,28,29,30,31 and 32]

### Epidemiology

There are marked differences in the geographic distribution of nasopharyngeal carcinoma. The incidence is approximately one case in 100,000 among the white population of North America, yet the incidence may be as high as 20 in 100,000 in Southeast Asia, 25 in 100,000 in Hong Kong, and 8 to 10 per 100,000 in North Africa. There may be an increased incidence of nasopharyngeal carcinoma in black teenagers in the United States. [30]

### Biology

Nasopharyngeal carcinoma is associated with Epstein-Barr virus (EBV) infection. [33,34,35,36,37 and 38] The EBV DNA may be demonstrated in malignant cells of biopsy specimens. These cells also express the EBV nuclear antigen. Markedly elevated antibody titers to various EBV antigens may be present, yet the greatest specificity and clinical importance are the titers of immunoglobulin A (IgA) and IgG antibodies to the viral capsid antigen. These titers usually correlate with the total tumor burden and decrease with successful therapy. They may also increase before the appearance of recurrent disease, and thus are a useful indicator of disease activity.

### Pathology

The World Health Organization Classification of nasopharyngeal carcinoma recognizes three subtypes: type I is squamous cell carcinoma; Type II is nonkeratinizing carcinoma, and type III is undifferentiated carcinoma. Most cases of children and adolescents are type III. Type I is more typically found in older adult patients. Types II and III are associated with elevated EBV titers, yet type 1 is not. Type II and III may be accompanied by inflammatory infiltrate of lymphocytes, plasma cells, and eosinophils, which are often abundant. Two histologic patterns have been described called the Regaud [25] and the Schmincke[26] type, with collections of epithelial cells surrounded by lymphocytes in connective tissue, or in which the tumor cells are intermingled with inflammatory cells. Both patterns may be present in the same tumor.

### Clinical Presentations and Patterns of Spread

Nasopharyngeal carcinoma generally originates in the fossa of Rosenmüller and metastasizes initially to cervical lymph nodes, which causes the usual presenting sign and complaint of patients. Other areas of spread include direct extension throughout the oropharynx with epistaxis, trismus, blockage of the Eustachian tubes, hearing loss, and extension into the base of the skull with nerve palsies ( Fig. 38-1). Distant metastases may be present in the lung, mediastinum, bones, and visceral organs.

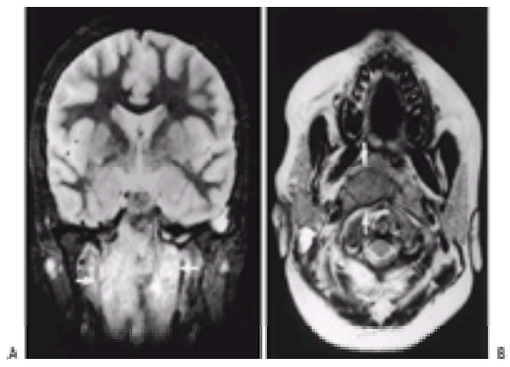

**FIGURE 38-1.** Coronal stir image **(A)** and transverse T2 image **(B)** of a nasopharyngeal carcinoma in a 12-year-old Hispanic boy. Images demonstrate large nasopharyngeal tumor (*arrows*) but do not demonstrate extension of tumor to the dura, which was found on additional views.

Cervical lymphadenopathy may be the only presenting symptom. The diagnosis is often made by lymph node biopsy. [39,40,41 and 42] Metastatic spread may result in bone pain or symptoms related to organ dysfunction at the sites of visceral metastasis. A paraneoplastic syndrome of marked osteoarthropathy with joint swelling, clubbing, and bone and joint pain may occur with widespread disease or with relapse. [43,44 and 45]

### Differential Diagnosis

Differential diagnosis includes other malignancies that may present with primary or secondary tumors in the nasopharynx. [44] Rhabdomyosarcoma and non-Hodgkin's lymphoma are the most frequent malignant tumors, whereas the most frequent benign tumor of this site is angiofibroma, [46] which usually presents with bleeding and is not associated with lymphadenopathy. Thyroid cancer may also present with significant cervical lymphadenopathy.

### Evaluation

The child with nasopharyngeal carcinoma should have studies to define the extent of disease and rule out distant metastatic spread. The size and location of cervical lymph nodes should be documented and indirect nasopharyngoscopy should be performed if the primary tumor is not obvious. Neurologic examination should focus on the cranial nerves. Computed tomography (CT) and magnetic resonance imaging (MRI) should include appropriate views of the skull and brain. CT evaluations of the chest and abdomen and a radionuclide bone scan should be performed to detect metastatic disease. If there is invasion of tumor through the base of the skull, cerebrospinal fluid examination should be considered to identify tumor cells. Bone marrow examination is unnecessary unless there is a strong suspicion of a process involving the bone marrow.

EBV titers should be assayed by a laboratory familiar with a full battery of the EBV serology, including monospot, EA, D titers, IgA and IgG anti–viral capsid antigen. [33,34,35,36,37,38 and 39] Direct consultation with the clinical laboratories is usually necessary to ensure the performance of the most important tests.

### Staging and Prognosis

The extent of the tumor at diagnosis is as described by the tumor, node, metastases (TNM) classification of the AJCC ( Table 38-1). [1,47,48] Because there is a high incidence of lymph node metastasis at diagnosis, most children and adolescents with this tumor are staged as having stages III and IV, yet these stages have little effect on planning of therapy and assessment of prognosis.

**TABLE 38-1. STAGING OF NASOPHARYNGEAL CARCINOMA[a]**

Various investigators have noted survival rates varying from 8% to 75%, with an overall 75% survival rate for T1 and T2 lesions, and 37% for T3 and T4 lesions. At St. Jude Children's Research Hospital, 78% of patients were disease-free with T1 or 2 lesions, with a 24% disease-free survival for patients presenting with T3 and T4 lesions. [49]

### Treatment

#### *Surgery and Radiation Therapy*

The nasopharynx is difficult to approach surgically. Because nasopharyngeal carcinoma generally has spread at the time of diagnosis, the principal role of surgery for therapy is to obtain adequate tissue for diagnostic purposes from an involved lymph node or the primary site. Other requirements may be myringotomy, or tympanostomy, if otitis media is present or expected. [50]

Involved areas are rarely accessible for resection after radiation therapy, which remains the primary therapeutic modality for nasopharyngeal carcinoma. The volume for radiation should include the nares; pharynx; posterior nasal cavity; posterior maxillary sinus; base of the skull, including the sphenoid and cavernous sinuses; and the cervical lymphatics, including the supraclavicular nodes. Recommended doses are 6,000 to 7,000 cGy. [41,51,52,53,54,55 and 56] Radiation therapy may be also given with curative intent for recurrent disease, locally or distally.

#### *Chemotherapy*

Most therapeutic trials for adults and children combine chemotherapy with irradiation. [51,52,53,54 and 55] These tumors and other head and neck cancers are unquestionably responsive to agents such as cisplatin, carboplatin, 5-fluorouracil (5-FU), methotrexate, and bleomycin. [51,52,53,54,55,56,57,58,59 and 60] At the present time the basic treatment regimens prefer to omit bleomycin. A multi-institutional study of this tumor in children and adolescents has demonstrated an excellent survival rate with the use of neoadjuvant cisplatin plus 5-FU and methotrexate/leucovorin given as four courses before irradiation. Twenty of twenty-one patients with advanced disease achieved long-term disease-free survival. [49]

#### *Complications of Treatment*

Xerostomia is a primary side effect of treatment. It may begin during radiation treatment along with mucositis. Sialadenitis may develop within a few months after completion of therapy. Other late effects include fibrosis of the neck and trismus. Muscle atrophy may be expected if children receive large doses of irradiation, and this may be associated with other problems, such as hypothyroidism, which require long-term routine follow up. Patients should continue to be evaluated for complications related to the chemotherapy and irradiation, including secondary tumors.

## AMELOBLASTOMA/ADAMANTINOMA

Ameloblastoma is also known by the term *adamantinoma*. [60,61,62 and 63] The ameloblastoma usually arises on the maxilla or mandible, and adamantinoma generally refers to tumors of long bones. The tumor may be benign or malignant, related to the development of the enamel of the teeth. Surgical excision is the treatment of choice, and no recognized chemotherapeutic regimen is used in an adjuvant situation. Local recurrence is more frequent for mandibular lesions than for maxillary lesions. [62] Sensitivity of recurrent tumors to irradiation is recognized; however, sensitivity to chemotherapy is unknown. Pulmonary metastases have been discovered many years after treatment of the primary tumors of the long bones. [63]

## SALIVARY GLAND TUMORS

The diagnosis and management of salivary gland tumors are complicated by their diverse nature and relative infrequency. [64,65] Most of these neoplasms originate in the parotid gland, with 10% to 15% arising from submandibular, sublingual, or minor salivary glands. These lesions may be primary or secondary after treatment with previously delivered radiation therapy. [66,67] Among the cancerous lesions in children, mucoepidermoid cancer is followed in frequency by acinic cell carcinoma, undifferentiated carcinoma, and adenocarcinoma. [68,69] These lesions must be differentiated from such tumors as hemangioma, mixed tumor, and other benign lesions.

Staging is that of the AJCC, with surgical removal being the treatment of choice when feasible. Additional consideration for irradiation therapy should be given for lesions that may not be completely resected.[70] Cisplatin-based therapy is most extensively used for these malignant lesions. Karyotypic abnormalities of these tumors has been reported,[71] as well as association with EBV.[72] Prognosis for patients with these tumors is generally good.

## CANCERS OF THE LARYNX

Both benign and malignant tumors of the larynx are rare.[73] Benign tumors may include polyps and papillomas in association with cough, hoarseness, dysphagia, and cervical lymphadenopathy. Rhabdomyosarcoma is the most common malignant tumor to involve this area (see Chapter 32). Guidelines established for the treatment of laryngeal carcinoma in adults should be used for children or adolescents with this tumor, and should include surgery, radiation, or both. Rehabilitative efforts should begin with pre-operative counseling. An electronic speech device may be used immediately after surgery; approximately 10% of patients develop satisfactory esophageal speech. The electrolarynx transmits sounds from the neck or mouth, with speech from the neck being more easily understood than oral speech. The efforts of the American Cancer Society are involved with rehabilitation, including information, support, and social outlets for pediatric patients, patterned after those for adults. Long-term attention should be directed toward thyroid size and function for survivors who have received radiation therapy.

There has been an increasing emphasis of the role of juvenile papillomatosis involving the larynx and the development of cancer.[73,74,75,76,77,78 and 79] Juvenile papillomatosis, even though it is a benign overgrowth of epithelial tissues, is induced to proliferate by infection with the human papilloma virus. This may affect primarily the larynx or other parts of the respiratory tract. Surgical removal of polyps is the primary therapy, yet refractory and recurrent cases may respond to cytotoxic agents, interferon,[74] or external beam irradiation. Malignant degeneration to squamous cell or epidermoid carcinoma often follows long-standing cases of juvenile papillomatosis. Prognosis for such patients is poor, but therapy should be attempted, depending on the extent of this disease, using wide surgical resection, radiation therapy, or chemotherapy with regimens commonly used for the treatment of adult head and neck cancer.

## BRONCHOGENIC CARCINOMA

Primary lung cancers are extremely rare in childhood. Among these rare tumors, most pediatric cases of bronchogenic carcinoma are undifferentiated or adenocarcinoma; squamous cell carcinomas also have been reported.[80,81 and 82] These tumors may occur in children of any age, but they are more usually found during adolescence. These tumors may be associated with papillomatosis.[83] Management of children with bronchogenic carcinoma should be according to reasonable adult guidelines with resection of operable tumors. Radiation therapy and chemotherapy may be of some benefit in unresectable cases.

## PLEUROPULMONARY BLASTOMA

A rare dysontogenetic tumor of childhood, pleuropulmonary blastoma can present as a pleural or pulmonary mass.[84] Histologically, pleuropulmonary blastoma of childhood differs from adult pulmonary blastoma because of its primitive and embryonic stroma, absence of a carcinomatous component, and potential for sarcomatous differentiation.[84,85] Dehner and colleagues have subclassified pleuropulmonary blastoma of childhood into three subtypes: type I is exclusively cystic, type II exhibits both cystic and solid components, and type III is a solid tumor without epithelial-lined cystic spaces.[84] Type I tumors appear to have a better survival; however, transition from type I to type III is possible.[86] The histologic origin of this malignancy is uncertain, but it is thought to be an expression of the somatopleural mesoderm or the thoracic splanchnopleure. Cytogenetic analysis of pleuropulmonary blastoma has revealed many abnormalities, including del(2)(q31;q33), del(9)(q22), and del(17)(p11.2).[87] Recurrent chromosomal abnormalities including trisomies of chromosomes 8 and 2 also have been reported in a few cases of pediatric pleuropulmonary blastoma.[88,89] Abnormalities of the p53 tumor suppressor gene, Wilms' tumor suppressor gene, and the putative second genetic locus for Wilms' tumor were not found in preliminary investigations.[87] Pulmonary cystic lung disease has been reported to be present at the time of pathologic diagnosis in 38% of cases.[84]

This tumor is strikingly associated with a familial history of cancer. Priest and colleagues[87] documented an association with other tumors or dysplasias either in the index case or in family members in 12 of 45 patients (approximately 25%). Diseases associated with pleuropulmonary blastoma included pulmonary cysts, cystic nephromas, thyroid adenomas, thyroid carcinoma, rhabdomyosarcoma, germ cell tumors, Langerhans' cell histiocytosis, medulloblastoma, synovial sarcoma, brain sarcomas, other pleuropulmonary blastomas, Hodgkin's disease, and acute lymphoblastic leukemia. These observations suggest that pleuropulmonary blastoma is a strong marker for familial disease.[87] The median age at presentation is 34 months.[84] Presenting symptoms are not specific and commonly include respiratory distress, fever, chest or abdominal pain, pulmonary infections, pneumothorax, cough, anorexia, and malaise. Therapy includes surgical resection with lobectomy or pneumonectomy and chemotherapy and radiotherapy.[84,90] Chemotherapy with commonly used agents for sarcomas, such as vincristine, dactinomycin, and cyclophosphamide, and other agents, such as doxorubicin and cisplatin, have been used in the preoperative and adjuvant settings with mixed results.[84,90] The use of radiotherapy is also controversial. The overall prognosis is poor: half of all patients treated die within the first 2 years after diagnosis.[84] Patients with mediastinal or pleural involvement have a significantly poorer clinical outcome. Recurrences are usually local or involve the brain (44% of cases) and the skeletal system.[87]

## THYMOMA

In pediatrics, 43% of mediastinal tumors occur in the anterior portion of the mediastinum, whereas in adults, 54% of mediastinal tumors occupy this anatomic location.[91] Primary malignant lesions involving the anterior mediastinum include lymphomas, germ cell tumors, carcinoids, carcinomas, thymolipomas, cysts, metastatic tumors, and thymoma, most of which can be confused because of the similarity of the gross and microscopic appearance.[91] Thymoma, an epithelial malignancy arising from the thymus gland, accounts for nearly half of all primary anterior mediastinal tumors in adults.[92] The epithelial cell is the cell of origin of thymomas and thymic carcinomas, whereas the lymphocytic component is considered benign.[93]

### Epidemiology and Symptomatology

Thymomas are rare in adults and in children. Fewer than 10% of thymomas occur in patients younger than 20 years, and they account for less than 15% of anterior mediastinal masses in this age group, with only about 30 cases described in the medical literature.[91,94,95] Thymomas usually present in the fourth and fifth decades of life without a clear sex predisposition. Nearly half of all adult patients are asymptomatic at the time of initial diagnosis, and the malignancy is discovered incidentally during imaging studies of the chest.[93] One-third of adult patients present with a variety of symptoms, including cough, chest pain, hoarseness, superior vena cava syndrome, and dysphagia. Approximately 30% of patients with thymoma have myasthenia gravis.[93,96] Conversely, 10% to 15% of adult patients with myasthenia gravis have an underlying thymoma.[96] In addition to myasthenia gravis, a variety of other paraneoplastic syndromes have been associated with thymoma. These syndromes occur in 5% to 10% of adults with thymoma and include pure red cell aplasia, hypogammaglobulinemia, and autoimmune or immune disorders such as scleroderma, dermatomyositis, systemic lupus erythematosus, rheumatoid arthritis, and thyroiditis.[93,96] Endocrine disorders associated with thymomas include hyperthyroidism, Addison's disease, and panhypopituitarism.[91,97,98]

### Pathology and Staging

Thymomas are generally located anterior to the great vessels of the mediastinum, which may be displaced posteriorly by the tumor. The masses are usually round with smooth or lobulated margins and may protrude to one or both sides of the mediastinum. Calcifications may be seen. The nomenclature for pathologic classification of thymic neoplasms is still evolving. Thymomas can be subdivided into three main categories: predominantly lymphocytic, mixed lymphoepithelial, and predominantly epithelial. It is well established that the epithelial cell is the malignant component of thymomas and that a predominance of this pattern is associated with a greater risk of invasion and a poor clinical outcome.[93,96] A prominent organoid pattern has been reported in pediatric cases.[99] The invasiveness of the tumor rather than the histologic architecture predicts clinical outcome.

Thymomas generally are slow-growing tumors. Although almost all are potentially invasive, metastasis to distant organs or regional lymph nodes is rare. Involved organs have included bone, liver, kidney, brain, spleen, and colon.

Appropriate evaluation of patients suspected of having thymoma or other mediastinal tumors includes chest radiographs and CT of the chest. MRI can distinguish vascular structures from tumor but does not offer a clear advantage over CT. The differential diagnosis of other anterior mediastinal masses includes Hodgkin's disease and non-Hodgkin's lymphoma, thymolipoma, carcinoids, germ cell tumors (e.g., primary germinoma, nonseminomatous and mixed germ cell tumors), and

thymic carcinomas. In the Mississippi Valley, histoplasmosis should be included in the differential diagnosis of mediastinal tumors.

The staging system devised by Masoka and colleagues as been widely adopted.[92,93] This postsurgical staging system classifies thymomas into two general categories: noninvasive (stage I) and invasive (stages II to IV). Invasive thymomas can be further subclassified into minimally invasive (II), extensively invasive (III), or metastatic (IV). Prognosis is highly dependent on clinical stage. Survival rates for stage I tumors range from 89% to 95%, whereas only 30% to 70% of patients with invasive thymomas are expected to be long-term survivors.[91,93,96]

**Treatment**

Surgery is the preferred modality for staging and treating patients with thymoma. The surgical incision of choice is a median sternotomy. [93] This approach allows adequate visualization of mediastinal structures and is relatively painless. An attempt should be made to resect all disease. Thymomas are usually staged at the time of initial surgical explorations because 30% of patients will have invasive disease at time of initial surgical exploration. [96,100]

For patients with completely resected encapsulated tumors, the intrathoracic failure rate after complete surgical excision is less than 5%; thus, adjuvant radiotherapy does not appear to offer a therapeutic advantage for these patients. [96]

Because thymomas are relatively radiosensitive,[101] radiation therapy is recommended for patients with invasive disease regardless of the degree of surgical resection. Patients with completely resected invasive disease who are treated with surgery alone have a 38% local failure rate compared to a 0% to 5% local failure rate when radiation therapy is added.[93,96] Dosage recommendations are based on the age of the child and the extent of invasiveness of the tumor. Dosages of 3,500 to 4,500 cGy delivered over 3 to 6 weeks are recommended for control of incompletely resected thymomas.[101]

Chemotherapy is generally reserved for patients with advanced-stage disease who have not responded to irradiation or corticosteroid therapy. Doxorubicin and cisplatin are recognized as effective agents for the treatment of this tumor, although responses also have been reported with alkylating agents. [93] The combination of cisplatin and etoposide has also proved active in patients with metastatic or recurrent disease. [102] The addition of ifosfamide to this regimen has produced similar results (50% response rate).[96] Taxol and carboplatin have been successful in selected cases with recurrent disease. [103] Preliminary results of a combined modality therapy trial using cisplatin, doxorubicin, cyclophosphamide, vincristine, and radiotherapy have shown a 77% response rate in patients with advanced-stage disease.[96]

Because thymomas have a high uptake of indium-labeled octreotide, trials using this somatostatin analogue have been recently conducted in patients with refractory disease. A durable complete remission has been noted in a patient with pure red cell aplasia and heavily pretreated thymoma using high-dose octreotide and prednisone.[104] In another trial, 7 of 13 patients with chemotherapy-refractory disease responded to prednisone and octreotide. [96]

In studies of adults and children at large institutions, 5-year survival rates ranged from 65% to 83% for encapsulated tumors and 30% to 54% for invasive tumors. The overall survival rate for patients with invasive thymoma and myasthenia gravis appears to have improved due to earlier detection of thymomas in this population.[91,105,106] and [107]

Complications of therapy may be related to the use of radiation. These complications may include pneumonitis, mediastinitis, pericarditis, and myocarditis.

## BRONCHIAL ADENOMAS

Most cases of bronchial adenoma are in fact carcinoid tumors or slow-growing malignancies such as mucoepidermoid carcinomas. [108,109] and [110] The primary treatment of these tumors is surgical resection. Other lung tumors that may enter into a differential diagnosis include leiomyosarcoma, primary or secondary rhabdomyosarcoma, myofibroblastic tumors, and hemangioendotheliomas. Bronchial adenomas,[108,109] and [110] also referred to as *mucoepidermoid tumors*, should be considered in the differential diagnosis of any patient with a persistent radiographic abnormality and clinical features simulating bronchial asthma. These low-grade epidermoid tumors generally do not metastasize but may be associated with cardiac disease [111] as well as secondary tumors.[112] Chemotherapy is generally unnecessary except in the presence of distant metastases.[113]

## MESOTHELIOMA

The pleural, pericardial, and peritoneal surfaces are the primary sites of mesotheliomas: the tunica vaginalis may also be a primary site. [114,115] and [116] These tumors may occur as primary or secondary malignant neoplasms and may be composed of epithelial, sarcomatous, and mixed histologies. [117,118] and [119] There is no widely accepted staging system for mesothelioma. It is, however, known that if these tumors are not treated, death usually results within 12 to 15 months from diagnosis. The determinations of which patients should be selected for surgery and chemotherapy are not understood. [120] Radiation therapy may be used for palliation of pain. Hyperthermia may enhance the effects of both radiation and chemotherapy. [121] One of our patients with a primary mesothelioma had a partial response with ifosfamide and another had long-term disease control with 5-FU plus leucovorin. High-dose methotrexate with leucovorin may be of some value. [122]

Many adults with mesothelioma have had a prior history of exposure to asbestos at industrial sites. Information about the risk for children exposed to asbestos is not available.

Secondary mesotheliomas have developed after malignant ovarian teratoma, Hodgkin's lymphoma of the neck, and non-Hodgkin's lymphoma (Burkitt's type) of the abdomen in three of our patients who had previous radiation and chemotherapy. [119] Prolonged survival was possible for one of these patients. Benign and malignant mesothelioma cannot be differentiated on histologic grounds. Poor prognosis is associated with diffuse or invasive lesions and those that recur.

## TUMORS OF THE HEART

Benign tumors are the most frequent of the cardiac tumors and may include myxomas and neurofibromas, tumors of muscle, or tumors of nerves.[123] Other tumors can include metastatic tumors such as melanoma, rhabdomyosarcoma, leukemia, and carcinoma of other sites.[124,125] The primary cardiac tumors may also include benign and malignant teratoma, hemangioma, chondrosarcoma, and rhabdomyosarcoma. Most symptoms include abnormalities of heart rhythm, enlargement of the heart, pericardial fluid, and congestive failure. Successful treatment requires surgery, which may include heart transplantation, and appropriate chemotherapy for the type of cancer being treated.[126]

## CANCER OF THE ESOPHAGUS

Cancer of the esophagus is rare in children and occurs more frequently in boys than in girls. Most tumors are epithelial in origin, represented by squamous cell carcinoma or its variants. Sarcomas of the esophagus are rare. The most common benign tumor of the esophagus is leiomyoma. Adult and pediatric patients with this cancer present with dysphasia, difficulty swallowing, and weight loss. There may be associated vomiting, cough, hemoptysis, hematemesis, regurgitation, and bone pain if there are metastases. Literature reviews of pediatric patients for this tumor appear in case reports from India. [127,128] The diagnosis must be made by histologic examination. Generally, barium-contrast radiography is followed by endoscopy and biopsy. A tissue biopsy should be obtained through endoscopy, with which there have been major strides in the diagnosis, staging, and treatment of esophageal cancer. [129] Early stage carcinomas of the esophagus are generally asymptomatic and may be detected incidentally.

The therapy for patients with esophageal cancer is based on the anatomical extent of the disease, as noted by the TNM classification. [1]

## CANCER OF THE STOMACH

Cancer of the stomach accounted for approximately 24,000 new cases in 1999 in adults.[1] Thus it remains an exceptionally rare cancer of children and adolescents but

remains one of the most common causes of cancer deaths in the United States.[140]

### Epidemiology

In 1936 gastric cancer was the leading cause of cancer-related deaths of men in the United States. The death rate and frequency of this disease have, however, declined worldwide since that time.[130] There are no recognized genetic syndromes associated with gastric cancer. Familial occurrence of this cancer is rare.

### Pathology

Approximately 95% of the tumors of the stomach are adenocarcinomas. Gastric adenocarcinomas are classified according to the degree of histologic differentiation. Approximately half of all stomach neoplasms are located in the distal stomach.[131,132,133 and 134] Nodal and omental involvement may be encountered. Other less frequent tumors include lymphomas, squamous cell carcinoma, carcinoids, leiomyosarcoma, gastrointestinal stromal tumors (GISTs), and gastrointestinal autonomic nerve tumors (GANTs).[135] Differential diagnosis includes Peutz-Jeghers–type polyps in the stomach, hemangioma, leiomyosarcoma, and liposarcoma. Gastric carcinomas spread by the lymphatics and blood vessels, by direct extension, and through seeding of the peritoneal surfaces. These lesions may infiltrate the submucosa, extend directly, and involve the duodenum or esophagus, liver, pancreas, or colon. Blood-borne metastases may involve the lungs, liver and skin. [136]

### Clinical Presentations

Cancers of the stomach produce vague epigastric discomfort, which may or may not be associated with weight loss and anorexia.[133,134,135 and 136] Iron deficiency anemia may be present with occult blood in the stool. These cancers may not produce symptoms until metastases are noted. For these reasons, individuals with weight loss and abdominal pain, nausea and vomiting, change in bowel habits, anorexia, dysphagia, weakness, hematemesis, or other vague abdominal symptoms should be investigated.

### Diagnosis

A biopsy through a gastroscope is the most accurate method of identifying gastric carcinoma. Fiberoptic endoscopy may be complemented with upper gastrointestinal series. Chest radiographs and CT scans should also be performed along with appropriate laboratory studies, including blood chemistry determination and complete blood cell count.

### Staging

The TNM classification is used for staging. This information is obtained from surgical exploration and from clinical data when resection is not carried out. Staging follows the latest AJCC Staging Handbook.[1]

### Prognostic Considerations

Prognosis for carcinoma of the stomach depends on the extent of disease and the treatment. There is little information about the outcome for patients younger than 21 years at the time of diagnosis and treatment.

### Treatment and Complications

Complete surgical excision with appropriate margins is the procedure of choice for this cancer. Surgery should include subtotal gastrectomy with resection of associated lymph nodes. The recommendation has been made that total gastrectomy not be performed unless there is potential for cure, because extended resection may be associated with increased mortality rate without adding to the likelihood of cure. The preferred methods for combining surgery and radiation for gastric carcinoma differ. Preoperative, intraoperative, and postoperative radiation have been given. [136] Although this tumor has been regarded as a somewhat unresponsive tumor, radiation therapy with curative intent is associated with tumor doses greater than the tolerance of surrounding tissues. The greatest benefits have been with combined radiation and chemotherapy after surgery. As expected, there are no data available on irradiation therapy for children.

Effective chemotherapy regimens do not exist for gastric carcinoma.[136] Adjuvant chemotherapy continues to be investigated. Among the agents being used are 5-FU without leucovorin, nitrosourea with or without doxorubicin, mitomycin, and irinotecan. Patients who respond to combinations of agents generally survive longer. There are no contemporary treatment data available related to results in children, and, as expected, complications may be related to the modalities used.

The effects of *Helicobacter pylori* infections during childhood and later gastric carcinoma are not defined. [137,138 and 139]

## PANCREATIC CANCERS

For adults, pancreatic cancer is the fourth most frequent cause of death, exceeded only by colon, lung, and breast cancers. [140,141] These carcinomas are among the most aggressive of the visceral malignancies and account for about 27,000 deaths in the United States annually. Pancreatic carcinoma is the seventh most common cancer in the United States.[140] Annual age-adjustedincidence rates for all carcinomas in patients younger than 20 years is 1.4 per million; thus, specific figures are unavailable for pancreatic cancers in this age group. Several cases of the usual adult type of pancreatic carcinoma have been reported for children, whereas most of the carcinomas in children are termed pancreatoblastoma.

There are no recognized genetic syndromes associated with pancreatic carcinoma in children or adolescents. [142,143,144,145,146,147 and 148] There is evidence to suggest that melanoma-prone families with mutations that impair p16 function may be at high risk of developing pancreatic cancer. [149] Although endocrine tumors of the pancreas may be associated with other hormone-producing tumors such as gastrinoma and insulinoma, congential pancreatoblastoma has been found in association with the Beckmann-Wiedemann syndrome.

### Pathogenesis, Natural History, and Patterns of Spread

The causes of pancreatic cancers in children are unknown. These tumors may arise in the head, body, or tail of the pancreas. [141] In most instances, the tumors are nonfunctioning, and symptoms differ according to the site of origin. Functioning tumors may produce a variety of symptoms. Islet cell carcinomas produce an overabundance of insulin, leading to hypoglycemia that may be associated with fatigue, restlessness, and malaise, followed by clouding of the sensorium, staggering gait, hyperthermia, and coma; these may appear as intermittent attacks, most frequently in the early morning hours. [142,143,144 and 145] Nonfunctioning islet cell tumors are usually associated with peptic ulcer and the elaboration of gastrin by the tumor; some patients develop watery diarrhea, hypokalemia, and achlorhydria.

The natural history of each of these tumors and of pancreatoblastoma is marked by wasting and pain. [143,146,147] Patients usually present with a large abdominal mass. There may be mechanical obstruction of duodenum and gastric outlet by tumors at the head of the pancreas, which may be associated with jaundice and intestinal hemorrhage. Venous obstruction may lead to varices, hemorrhage, and ascites. Tumors of the body or tail of the pancreas may erode into the stomach and cause hemorrhage. Ascites associated with involvement of the liver and peritoneum may result in hepatic failure, and patients may die because of progressive weight loss and anorexia.

### Diagnosis

With the objective of establishing diagnosis and extent of disease, the usual diagnostic imaging studies should be used along with evaluation of the chest by CT. Differential diagnosis should include benign neoplasms such as papillary cystic tumor and hemangiomas. Radiographic studies should include contrast studies of the gastrointestinal tract, abdominal ultrasonography, and CT or MRI scans of the abdomen. Primary tumors of the head of the pancreas may cause deformity of the duodenal-C loop, or gastric antrum, and may be associated with mucosal abnormalities detected at gastroscopy. Retrograde endoscopic cholangiopancreatography

may be helpful, as may be arteriography for patients being considered for resection.

Serum markers including carcinoembryonic antigen, alpha-fetoprotein, CA19-9, and pancreatic oncofetal antigen may be of value in the diagnosis and in follow-up.[150,151] Other markers such as amylase, lipase, alkaline phosphatase, lactic dehydrogenase, transaminase, leucine aminopeptidase, and pancreatic ribonuclease may aid in diagnosis and determination of the success of treatment.

### Pathology

Pediatric malignant tumors of the pancreas, in descending order of origin, include malignant papillary cystic carcinoma [152] and pancreatoblastoma[152,153,154,155,156 and 157] as well as tumors of islet cell origin such as insulinoma and gastrinoma. [158,159 and 160] Other cancers of duct cell origin are adenocarcinoma and squamous cell carcinoma; acinic cell carcinoma, liposarcoma, and lymphoma may involve the pancreas.

Surgical staging for this tumor uses the grading system of the AJCC, and the reader is referred to the most recent edition of that handbook. [1]

### Prognostic Considerations

Fewer than 10% of adult patients with carcinoma of the pancreas survive, yet better results have been observed in children. Most patients in the pediatric age group who develop this rare type of carcinoma die, usually because the diagnosis may not be made until after the development of locoregional disease. Most tumors of the pancreas are not radiosensitive or chemosensitive, thus the only potentially curable patients are those diagnosed at an early stage when the tumor is confined to the body of the pancreas. For these individuals surgical resection provides the only chance for long-term disease-free survival. Pancreatoblastoma may defy these statements.

### Treatment

The principles of treatment for pediatric patients with pancreatic tumors have been derived from the adult experience. [141,158] Various operations that have been developed since 1935 include pancreatoduodenectomy, total pancreatectomy, regional pancreatectomy, and distal pancreatectomy. The standard operation for resection is a pancreaticoduodenectomy, referred to as the *Whipple procedure*. Too few pediatric patients have been treated with radiation therapy for pancreatic carcinoma or pancreatoblastoma to make meaningful data available. For adults the treatment dosage has been 4,500 cGy over 4 to 6 weeks. Complications may include bowel obstruction, biliary obstruction, and biliary fistula. Studies for adults are presently ongoing to evaluate the use of adjunctive conventional or intraoperative radiation therapy.

### Localized Tumors

Chemotherapy for pancreatic carcinoma varies from that given for pancreatoblastoma. The agents for metastatic pancreatic carcinoma include 5-FU, streptozotocin, mitomycin C, gemcitabine, and doxorubicin, associated with response rates of 7% to 36%. [141,161,162 and 163] For pediatric patients with pancreatoblastoma, the usual agents include vincristine, cyclophosphamide, doxorubicin, cisplatin or carboplatin, dactinomycin, or bleomycin. [153,155,157] Surgical excision and radiation therapy are necessary after obtaining chemotherapy response in unresectable lesions to prevent regrowth of tumors. [162,163]

## GASTROINTESTINAL STROMAL TUMORS

Although these tumors occur in middle-aged and older individuals and are rare before the age of 40 years, recent evidence is that more tumors are being classified into this category than in the past. Such tumors as leiomyosarcoma and synovial sarcoma have in some instances been reclassified as GISTs. [164]

These tumors may arise at any place in the gastrointestinal tract and are histologically different from true leiomyosarcomas, leiomyomas, leiomyoblastomas, and malignant tumors of peripheral nerve sheath. Greater than one-half of these tumors arise in the stomach and have spindle cell as well as epithelioid characteristics. Other sites include the esophagus and the small intestine. [166]

A group of malignant small intestinal tumors, sometimes referred to as plexosarcomas, have been termed *GANTs*.[165,166] These tumors were histologically heterogenous spindle cell or epithelioid tumors of stomach, small intestine, mesentery, and retroperitoneum. These tumors have been reported in patients with neurofibromatosis type I.

Nearly all GISTS have expressed the C-kit gene that is located on the long arm of chromosome 4. [167] This genetic mutation is not found with true smooth muscle tumors including leiomyomas and leiomyosarcomas. Chromosome deletions have been reported on the 9p and 22q preferentially in malignant GISTs. [167]

The GANTs[164] are characterized by ultrastructural features resembling autonomic nerve cells, without epithelioid, Schwannian, or smooth muscle differentiation. In children there may be a prevalence in females in the second decade with predominance of smaller gastric tumors and the possibility for more significant prognostic value related to age. In the past these tumors have been treated with the usual agents for soft tissue sarcomas; as soon as possible after surgery some tumors require the use of chemotherapy or radiation therapy, with agents used for sarcomas.

## RENAL CELL CARCINOMA

Renal cell carcinoma (e.g., clear cell carcinoma, renal cell adenocarcinoma, hypernephroma) was first studied by Grawitz, [168] who believed that the tumor arose from ectopic adrenal tissue because of its microscopic similarity to adrenal cells. Because of this similarity, he named this tumor *hypernephroma*, a misnomer that is occasionally still used.

Renal cell carcinoma is the most common primary malignancy of the kidney in adults, accounting for 2% of adult cancer cases. [169] In children, the overwhelming majority of renal malignancies are nephroblastomas, and only 7% of all primary renal tumors in patients younger than 21 years are renal cell carcinomas. [170] Referral patterns to the Armed Forces Institute of Pathology seem to indicate that the incidence of renal cell carcinoma may approach that of Wilms' tumor in the second decade of life.[171,172] Although renal cell carcinoma in adults occurs with a male to female ratio of 2:1, childhood series show a slight female predominance. [170,173,174]

### Risk Factors

Almost all of the information regarding risk factors in renal cell carcinoma have been derived from case-controlled studies. In adults, cigarette smoking, obesity in women, and renal dialysis have been linked to an increased incidence of renal cell carcinoma. [169]

Renal cell carcinoma occurs in both sporadic and familial forms. Von Hippel-Lindau disease (VHL) is a dominantly inherited disorder characterized by germline mutations of the tumor suppressor gene VHL, which is located on chromosome 3p25.[175] This syndrome is characterized by retinal angiomas, central nervous system hemangioblastoma, multiple renal cysts that grow slowly over years, and the development of renal cell carcinoma. The latter can be multicentric or bilateral, usually of the clear cell histologic subtype. Renal cell carcinoma can develop in the preexisting cysts or de novo and has been reported to occur in up to 60% of patients with VHL.[175] In addition,VHL is also associated with the development of pheochromocytomas, pancreatic cysts, islet cell tumors of the pancreas, endolymphatic sac tumors, and papillary cystadenomas of the epididymis and adnexal tissues in women.

Tuberous sclerosis, a disorder characterized by multiple hamartomas, renal angiomyolipomas, seizures, and learning disabilities has also been associated with the development of renal cell carcinoma of the clear cell type. [175] Familial renal cell carcinoma has been associated with a constitutional chromosomal translocation, t(3:8)(p14;q24). The *TRC8* gene, which maps to the 8q24 breakpoint, appears to play a major role in the genesis of renal cell carcinoma of the clear cell type in these patients.[175,176] Chromosome studies of nonfamilial tumors have shown a high incidence of abnormalities of chromosome 3. Hereditary papillary renal cell carcinoma has been reported in several families. Germline *MET* proto-oncogene mutations have been reported in as many as 80% of cases.[175] In children,VHL gene

abnormalities are very rare. There is a growing body of evidence that translocations or deletions involving the Xp11.2 loci define a subgroup of younger children with papillary renal cell carcinoma. A specific chromosomal translocation, t(X;1)(p11;q21), which fuses the *TFE3* and *PRCC* genes, has been recently identified.[177]

### Pathology and Patterns of Spread

Microscopically, the tumor tissue resembles renal tubules. Four major patterns of growth have been described: papillary, solid, cystic, and sarcomatoid. The papillary subtype predominates in some series of children with renal cell carcinoma.[178] There is apparently little prognostic significance to these patterns, and in some cases, several patterns can be found within the same tumor. In addition, three different types of cellular morphology may be identified: clear cells, granular cells, and sarcomatoid cells.

Renal cell carcinoma typically metastasizes by hematogenous and lymphogenous spread. Lungs, bones, liver, lymph nodes, and the mediastinum are common metastatic sites.

### Clinical Presentation

Unlike in children with Wilms' tumor, which often presents as an abdominal mass, children with renal cell carcinoma typically present with abdominal or flank pain, gross hematuria, or both. A palpable mass is apparent in only 60% of cases[179] and often must be demonstrated by ultrasound or CT. Calcifications are seen in 24% to 50% of the cases.[179,180 and 181] A variety of paraneoplastic syndromes have been reported in adults, including hepatic dysfunction that resolved after resection of the primary tumor.[182] Other syndromes are caused by ectopic production of various hormones including parathormone (hypercalcemia), erythropoietin (polycythemia), gonadotropin (gynecomastia), and various other substances.[183,184] These syndromes have not been a notable feature of renal cell carcinoma in childhood.[173]

Children with renal cell carcinoma are typically older than children with Wilms' tumor, with a median age at diagnosis of 11 years.[185] However, the diagnosis has been made in children as young as 14 months.

### Differential Diagnosis

The obvious lesion to be considered in the differential diagnosis is Wilms' tumor, the most common renal neoplasm in childhood (see Chapter 28). Beckwith pointed out the occasional difficulty in differentiating Wilms' tumor from renal cell carcinoma, because some tumors have histologic characteristics that may be transitional between the two diagnoses.[172] Also to be considered are other space-occupying lesions of the pediatric kidney, including multilocular cysts, nephroblastomatosis, angiomyolipoma, congenital mesoblastic nephroma, benign stromal tumors, intrarenal neuroblastoma, renal teratoma, malignant rhabdoid tumor, clear cell sarcoma, lymphoma (especially Burkitt's), and renal sarcomas (e.g., rhabdomyosarcoma, liposarcoma).

### Evaluation

Renal cell carcinoma should be considered as part of the differential diagnosis, particularly in older children who present with pain or hematuria and a renal lesion. Studies to be obtained include a complete blood count (to evaluate anemia, erythrocytosis), biochemical profile (to evaluate hypercalcemia), renal ultrasound with attention to the patency of the inferior vena cava, CT of the chest, abdominal CT or MRI with and without intravenous contrast infusion, and a bone survey and radionuclide bone scan.

### Staging

Renal cell carcinoma in childhood has occasionally been staged according to the National Wilms' Tumor Study criteria. Although pediatric oncologists are most familiar with this staging system, it seems more appropriate to use one of the staging systems developed specifically for this tumor in adults. The TNM classification provides the most accurate description of disease extent and stratifies patients into four stages based on the size of the tumor (less than or equal to 7 cm vs. greater than 7 cm). It also takes into account whether the tumor is confined to the kidney or invades vessels, Gerota's fascia, perinephric tissues, and lymph nodes, and whether distant metastases are present.[1,186]

### Prognostic Considerations

The stage at diagnosis is the most important prognostic factor. Table 38-2 summarizes the experience in 93 children reported in seven series.[170,173] The long-term survival rates approached 100% for patients with tumors less than or equal to 7 cm and confined to the kidney (stage I). Patients with tumors larger than 7 cm or those with tumors that extended beyond the kidney or had lymphatic metastases to a single regional node (stage 2 and 3) had an intermediate prognosis, whereas patients with metastatic disease fared very poorly (only 1 of 32 survived).[174,185] Although other variables, such as tumor grade and renal vein invasion, correlate with stage at diagnosis, they do not appear to be independent prognostic variables. Review of 84 children with renal cell carcinoma reported actual survival rates of 56% and 50% at 5 and 10 years, respectively.[181] Most recurrences and deaths occur within the first 2 years after diagnosis, but late recurrences are not infrequent.

| Stage | Number of patients | Total number of patients/survivors (%) |
|---|---|---|
| I | 27 | 27/26 (96) |
| II | 15 | 15/9 (60) |
| III | 19 | 19/13 (68) |
| IV | 32 | 32/1 (3) |
| Total | 93 | 93/49 (53) |

**TABLE 38-2. CLINICAL CHARACTERISTICS AND OUTCOME OF 93 CHILDREN WITH RENAL CELL CARCINOMA**

### Surgery

The primary therapy of localized renal cell carcinoma is radical nephrectomy, with resection of the kidney and tumor, the adrenal gland, surrounding perinephric fat, Gerota's fascia, and the regional lymph nodes. Complete retroperitoneal node dissection should be reserved for large tumors (greater than 7 cm) when metastatic lymph nodes are documented. Low-stage tumors can be treated with partial nephrectomy using laparoscopic techniques. The local recurrence rate in this setting is 1% to 3%.

### Radiotherapy

The role of preoperative or postoperative radiotherapy in the management of renal cell carcinoma is much less clear than that of surgery. Although some series of adult patients have found enhanced survival with the addition of radiotherapy, other randomized studies have found no difference in outcome between treated and untreated groups. This situation is even more ambiguous for children, in whom renal cell carcinoma is a much rarer disease. Empiric administration of postoperative radiotherapy (4,000 to 4,500 cGy in 150-cGy fractions) has been recommended for children with stage III disease.[174] Radiotherapy can provide significant palliation by decreasing pain, mass effects, or hematuria from unresectable primaries or metastatic disease.

### Chemotherapy

No single chemotherapy agent or combination of agents has yet proved to be of significant benefit to the majority of patients with advanced disease. The combination of gemcitabine and 5-FU has produced responses in 17% of patients with metastatic disease. [187,188] Renal cell carcinoma may prove amenable to therapy with biologic response modifiers. Numerous trials have demonstrated complete or partial responses in 15% to 20% of patients treated with interferon-alfa and interleukin-2 (IL-2).[189] High-dose IL-2 has produced objective responses in 15% of patients. Of interest, the patients who achieve a complete response to IL-2 (approximately 7%) appear to have durable responses. [189,190] Combination therapy using a variety of biologics and chemotherapeutic agents, including interferon, IL-2, 13- *cis*-retinoic acid, floxuridine, and vinblastine, have also been performed.

Fortunately, at least half of pediatric patients with renal cell carcinoma are curable by surgery alone. Adjuvant radiotherapy remains controversial but should be considered when the disease has extended beyond the renal fossa. There is no satisfactory chemotherapy for renal cell carcinoma, but further refinement and development of immune and biologic therapies may provide a significant alternative for the treatment of this disease. For example, a recent report has documented regression of human renal cell carcinoma in seven patients who were treated with hybrid cell vaccination using monocyte-derived dendritic cells as fusion partners to tumor cells.[191]

## COLORECTAL CARCINOMA

Although colorectal carcinoma is one of the most frequent tumors of adults, it rarely occurs in individuals younger than 20 years. [192,193]

### Epidemiology

Colorectal carcinoma is rare in populations with limited meat intake. Thus it is more common in the West than in Africa. [194,195] It is thought to be related to the long transit time of fecal material in the bowels of persons consuming relatively low-fiber diets.

There are approximately 150,000 new cases of colorectal carcinoma in adults annually. [192,193] The Surveillance, Epidemiology, and End Results program data suggests that only about 80 cases occur in patients younger than 20 years. [193] Thus, the incidence is approximately one case per million persons in this age group.

For children and adolescents these tumors may occur in any site in the large bowel and are not usually associated with a family history of large bowel cancer.[196,197,198,199,200,201,202,203,204,205,206] and [207] There had been suggestions of an increased incidence of ovarian cancer in the families of younger patients with colorectal carcinoma.[207] There is no gender predilection for cancer, with most cases that occur in patients younger than 20 years centering around the age of 15 years.[203,204] and [205] Most of the case reports have come from the central Mississippi Valley, in which some patients had been exposed to pesticides and herbicides. [203] Long-standing ulcerative colitis patients are at increased risk, which increases with the duration and severity of colitis, with this risk increasing to approximately 20% after the age of 40 years.[208] There is a shifting incidence of the sites of primary lesions from the left to the right colon. [209] Black Americans have an increasing occurrence of colorectal cancer.[140,209]

### Genetics

There are several recognized conditions that may be associated with the development of colorectal carcinoma in young patients. These include familial adenomatous polyposis, inherited as a dominant trait with 90% penetrance, which may be associated with the appearance of multiple cancers by the age of 37 years. [210,211] Early diagnosis and colectomy can eliminate the risk of development for these patients. Other syndromes associated with colorectal carcinoma in young people include Turcot's syndrome, for which the frequent mutation of the adenomatous polyposis coli gene has been found; Oldfield's syndrome; and Gardner's syndrome ( Table 38-3).[213,216] There may be an association of neurofibromatosis and polyposis coli, and one individual with multiple adenomatous polyps and multiple colonic carcinomas had a deletion of the p53 gene, also in association with neurofibromatosis. [214,217,218] and [219]

| Name | Description | Reference |
|---|---|---|
| Gardner's | Osteoma, polyposis, multiple sebaceous cysts | 200 |
| Peutz-Jeghers | Mucocutaneous pigmentation of lips, perioral region, buccal mucosa, polyposis, ovarian granulosa cell tumors | 201 |
| Turcot's | Congenital brain tumors | 202–204 |
| Oldfield's | Multiple sebaceous cysts, polyposis | 205 |
| Rendu-Osler-Weber | Hepatic telangiectasia, juvenile polyps | The authors' unpublished observations |

**TABLE 38-3. POLYPOSIS SYNDROMES ASSOCIATED WITH COLORECTAL CARCINOMA**

The genetic events associated with the development of colorectal carcinoma have been elucidated by Vogelstein and colleagues [220] and others.[221,222] The familial adenomatous polyposis syndrome is caused by a constitutional mutation of a gene on chromosome 5. These investigators hypothesized that the inherited gene defect in familial adenomatous polyposis gives rise to widespread epithelial proliferation, which precedes the development of adenomas in these patients. Mutations of a kRAS gene on chromosome 12p and deletions of the tumor suppression gene 253 are also associated with the development of colorectal carcinoma. The loss of another tumor suppressor gene located on chromosome 18, dcc (deleted in colon cancer), is associated with progression of intermediate to late adenoma. For children and adolescents there is no evidence that a family history of bowel cancer confers a greater risk for the development of bowel cancer before the age of 20 years. The same is true for persons younger than 20 years belonging to families with hereditary colorectal carcinoma, cancer family syndromes, or familial juvenile polyposis (Fig. 38-2). Individuals with the Peutz-Jeghers syndrome may develop bowel cancer because of the risk associated with polyposis involving the upper and lower gastrointestinal tracts.[213]

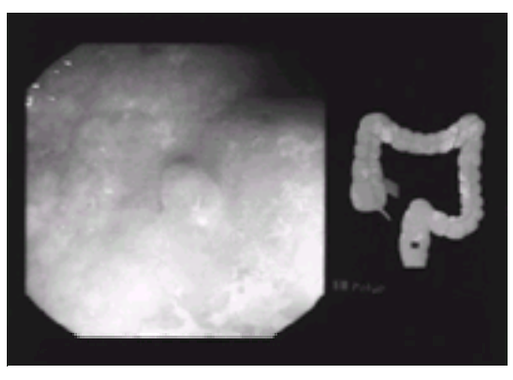

**FIGURE 38-2.** Endoscopic photograph of an adenomatous polyp in the rectum of a 20-year-old man. He had bloody stools and pain before diagnosis of a stage III mucinoses carcinoma of the cecum in 1987 at the age of 10 years. Additional polypectomies were performed in 1988 (before colon resection to the peritoneal reflection in 1989). The figure demonstrates a polyp seen in 1997.

## Biology

Signs and symptoms may be absent or innocuous. A change in bowel habits, such as constipation or diarrhea, and change in the caliber of stools may be observed before the development of tarry stools, rectal bleeding, or other changes in bowel habits. There may be a decrease in appetite and weight loss.

The signs and symptoms of colorectal carcinoma are related to its primary site within the large bowel. [193] Tumors involving the cecum and descending colon, which may be associated with familial colon carcinoma, may develop large masses before symptoms appear. Tumors of the rectum and sigmoid may be associated with changes in the caliber of the stool, dyschezia, and hematochezia.

The diagnosis of colorectal carcinoma in young individuals is often delayed because it is seldom suspected. Acute bowel symptoms necessitate immediate abdominal exploration, at which time perforation of the large bowel may be observed with multiple metastatic deposits. Intestinal obstructions due to tumor occur more frequently in adolescents than in adults with this cancer. [202,205,206]

The natural history of colorectal carcinoma in young individuals differs from that of adults, with tumors of the young being more advanced at diagnosis. [194,196] These tumors, therefore, may not be resected in early stage and may spread throughout the peritoneal cavity to involve the omentum, peritoneum, mesenteric lymph nodes, liver, and ovaries, and spread through the bloodstream to the lungs and eventually the brain, bones, or both. Peritoneal seeding in females frequently involves the ovaries, which may reach tremendous size due to tumor involvement. Greater than one-half of the neoplasms of the colon in younger patients are mucinous adenocarcinomas, which occurs in approximately15% of adults with colon carcinoma. [223]

## Ancillary Clinical Studies

Examination of the stool for occult blood may produce positive results when no gross blood can be seen on the stool or discoloring the stool. Hepatic and renal function are seldom contributory, as are studies of the urine. Hepatic function abnormalities may be related to metastatic involvement of the liver. Anemia may be related to blood loss or malnutrition. Carcinoembryonic antigen levels should be determined, yet fewer than 75% of the colon carcinomas in children may produce this protein. The frequency and positivity of this assay increase with increasing stage of disease.

## Imaging Studies

Direct or fiberoptic colonoscopy should be useful in locating the site of any lesion of the large bowel. The entire length of the colon should be evaluated, especially to note any association with polyps. Conventional radiographic studies include barium enema with air contrast to define the tumor and the remainder of the colon. These radiographs, performed in association with CT scans of the abdomen and chest, may define areas of spread to the liver, lungs, or enlarged lymph nodes, and metastases that may involve the pelvis and cul de sac, especially the ovaries. Radioisotope studies should also include a bone scan. If the bone scan is positive, a bone marrow aspiration or biopsy may be appropriate to determine if there has been spread to the marrow.

## Pathology

Colorectal carcinoma arises from the mucosal surface of the bowel, generally at the site of an adenomatous overgrowth of polyp. Tumor may extend into the muscularis area to the serosa and perforate the serosa into the omental fat, lymph nodes, liver, ovaries, and other loops of bowel. Some lesions may obstruct the bowel lumen. There also may be implants along the abdominal scar, at the anastomotic site, or throughout the peritoneum. Rarely is more than one cancer present simultaneously. Multiple lesions may have the same or different histology and may have the same or different stages of development. Carcinoma *in situ* may occur in one or more polyps and may be associated with the prior delivery of radiation therapy. Synchronous primaries have the same prognosis as single colon cancers. [228]

The gross appearance of colonic lesions depends on the extent of involvement of the lumen of the bowel and the extent of the disease outside the bowel wall. Because these tumors are derived from endoderm, all the cytologic characteristics will be that of carcinoma, yet they may be well-differentiated or poorly differentiated and contain pools of mucin. These tumors may grow to huge sizes, and in females, ovarian involvement may be massive and may result in difficulty in the differential diagnosis between bowel cancer and ovarian cancer.

The differential diagnoses include malignant carcinoid, leiomyosarcoma, malignant fibrous histiocytoma, and metastatic tumor from other sites. All may have similar presentations; metastases may be identified only by histologic or metastatic site. When individuals with this tumor present with an acute abdomen with associated pain and possible perforation, the diagnosis of acute appendicitis is most often considered.

## Staging

Staging systems have been proposed over the past 50 years for this tumor. The TNM Classification of the AJCC has been most frequently used for definitions for reporting requirements of tumor registries (Table 38-4). The staging system of the National Institutes of Health Consensus Development Conference reflects these definitions as well as the Dukes' Classification. [225]

**TABLE 38-4. STAGING OF COLORECTAL CARCINOMA**

## Treatment

General surgical principles: Biopsy is required for the diagnosis of colorectal carcinoma. [192] A biopsy may be obtained by colonoscopy or at laparotomy, at which time definitive surgery may or may not be feasible. Surgical staging procedures include biopsy of any known enlarged lymph nodes, biopsy of the ovaries in female patients, resection of the omentum, and biopsy of the liver. Complete excision is the goal for the surgeon, with secondary aims being related to palliation by resection of bulky tumors or metastases. Debulking provides little for the patient with extensive metastatic disease. Removal of single or multiple hepatic metastases may become a life-saving procedure for patients who had excision of the large bowel, nodal dissections, and metastases to the liver. Decisions regarding colostomy remain the prerogative of the surgical oncologist.

### *Chemotherapy and Radiation Therapy*

Tumor involvement of the rectosigmoid area or anus that are considered unresectable at the time of diagnosis should be treated initially with radiation therapy before

any surgical procedure other than biopsy.[192] Chemotherapy options are expanding from the use of 5-FU with leucovorin.[226,227] The use of interferon alfa-2A with 5-FU has been associated with significant intolerance of pediatric patients to interferon, and this treatment for adults was not associated with an increase in the survival rates for individuals with stage III or IV disease.[204,227,228] Irinotecanhas also been used in combination with 5-FU and leucovorin.[229,230] This topoisomerase inhibitor has significant activity in human xenografts as well as patients in phase I and II studies. Studies from France and Japan have indicated the activity of oxaliplatin for colorectal carcinoma.[231]

Intraoperative radiation therapy has been advocated for disease known to have metastasized to the mesentery or mesenteric lymph nodes. This procedure is performed while the bowel is displaced from the peritoneal cavity.

### *Complications of Therapy*

Complications of therapy are sometimes difficult to separate from the complications of the disease, which may be nutritional or obstructive or may be related to the effects of metastatic disease on other organ systems. Patients who have survived colon carcinoma may be at increased risk for development of secondary leukemias.[232]

### *Therapeutic Trends*

Current therapies are unsatisfactory for patients with stage III and IV tumors because these tumors are detected after the development of extension of disease. Clinical suspicion is not raised for the teenager with diffuse abdominal discomfort or mass. Surgery is the only modality known to be effective in providing cures, although adjuvant chemotherapy extends life. Few patients with extensive metastatic disease are cured. Irradiation of pulmonary or brain metastases may provide therapeutic benefit and symptomatic relief of the sequelae of these metastases.

Refinement of tumor cloning assays for prediction of response to treatment may be of significance for individuals with this tumor. Specific histologies may respond to varying chemotherapeutic agents.

For individuals at high risk of developing primary or recurrent colorectal carcinoma, annual colonoscopy has been recommended. For children, this probably should not be performed except at biennial intervals. Screening for fecal occult blood has not proved to be of significant value for the treatment and management of pediatric patients.

Recent studies have noted high frequency of microsatellite instability in 17% of 607 patients with colorectal carcinoma.[233,234] and [235] This was associated with significant survival advantage independent of all standard prognostic factors including tumor stage, and individuals with this occurrence had decreased likelihood of their tumors metastasizing to regional lymph nodes.[234,235] Microsatellite instability is a characteristic pattern of genetic instability, which occurs in microsatellite DNA that occurs in most hereditary nonpolyposis colorectal cancers. Studies at Memorial Sloan-Kettering Cancer Center on 29 patients younger than 21 years noted that tumors appear to develop by means of two pathways, either involving a tumor suppressor gene or loss of heterozygosity and other features involving a mutation.[233,234,235,236,237,238,239,240,241,242] and [243] Other genetic and developmental factors may account for the aggressive course of this disease.

The chemopreventive effects of nonsteroidal antiinflammatory agents on colorectal carcinoma involve the interaction of the adenomatous polyposis coli tumor suppressor pathway and the nuclear hormone receptor peroxisome proliferator–activated receptor gamma.[241,242,243,244,245] and [246] Both the number and size of colonic polyps was reduced in patients who were receiving aspirin and other nonsteroidal antiinflammatory drugs.

## PAPILLARY SEROUS CARCINOMA OF THE PERITONEUM

Papillary serous carcinoma of the peritoneum is an unusual tumor that may arise on the surface of the ovaries and spread to the omentum, abdominal, and pelvic peritoneum.[247,248,249,250] and [251] This tumor rarely affects adolescents and significantly resembles papillary carcinoma of the ovaries seen in adults. This tumor may be derived from Müllerian cells. The presenting signs are abdominal distension and pain. Treatment usually consists of debulking and cisplatin-based therapy, with or without paclitaxel. The prognosis for survival is poor, yet individuals may have an indolent course with recurrences over many years. Differential diagnosis must include mesothelioma of the peritoneum and surface carcinoma of the ovary.

## CARCINOMA OF THE BLADDER

Primary tumors of the bladder are rare in childhood. The most frequent of bladder cancers in pediatric patients is rhabdomyosarcoma (see Chapter 32). The most common carcinoma is transitional cell carcinoma, which may be encountered as a second malignant neoplasm after extensive treatment with cyclophosphamide.[252,253] and [254] Diagnosis and treatment are the same for children and adolescents as they are for adults.

## CERVICAL, VAGINAL, AND VULVAR TUMORS

Tumors of the cervix, vagina, and vulva are extremely rare in children and adolescents.[254,255,256,257,258] and [259] Rhabdomyosarcoma is the most common tumor of these sites, yet squamous cell tumors may also occur. Cervical squamous cell carcinoma has been observed more frequently with increasing age through adolescence.

Clear cell carcinoma of the vagina and cervix has been observed in the daughters of mothers who received diethylstilbestrol for the prevention of spontaneous abortion. Other abnormalities have included vaginal adenosis.[260,261,262,263,264] and [265] Treatment for the clear cell adenocarcinoma of these sites requires vaginectomy, hysterectomy, and lymphadenectomy. The role of radiation is not clearly defined. Other tumors of these sites include carcinomas, papillomas, nodular fasciitis, and sweat gland tumors. Principles for their treatment depend on the site, stage, and pathologic features.

## CANCERS OF THE BREAST

Although breast tumors of children and adolescents are usually benign, carcinomas have been reported. Primary malignant tumors of the breast include carcinomas, yet other tumors such as rhabdomyosarcoma and lymphoma may originate in the breast (Fig. 38-3).[266,267,268,269,270,271,272,273] and [274] Breast carcinomas may affect males and females. Recommendations regarding radiation therapy and chemotherapy for childhood and adolescent breast carcinoma are unavailable because no large contemporary series has been reported.

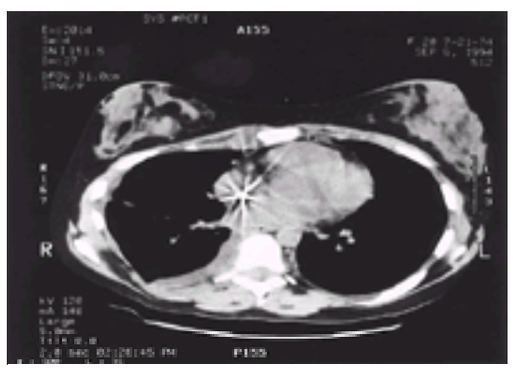


**FIGURE 38-3.** Axial noncontrast computed tomographic scans of thorax demonstrating multiple bilateral breast metastases from alveolar rhabdomyosarcoma in a 20-year-old woman. Film also demonstrates right pleural effusion and erosion of a right posterior rib. Primary site was paraspinal.

Another category that concerns pediatric oncologists are metastatic tumors that may involve the breasts. [272,273] This has been seen more commonly in individuals with alveolar rhabdomyosarcoma of other sites. Another category of tumors of the breast includes secondary neoplasms, which include carcinomas. These tumors generally follow radiation therapy delivered to the chest for such diseases as Hodgkin's disease or cystosarcoma phylloides. [274] Giant fibroadenomas are also tumors that may occur after puberty. These tumors may be slow in growth and not suggest malignancy; most are benign, present as a mass, and should be excised.

Epidemiology studies of breast cancer and aggregation in families have been commented on in Chapter 2 in relationship to the Li-Fraumeni syndrome. [275,276]

## CARCINOID TUMORS

Carcinoid tumors are rare; they are of epithelial origin and may be benign or malignant. [277,278,279,280,281,282,283,284,285 and 286] Female predominance has been noted with this tumor. They may be located in the esophagus or bronchi, or in the large or small bowel, appendix, pancreas, or ovary. On occasions a primary site cannot be discerned. The most common site is the appendix, in which most tumors are benign. [281,282,284] Approximately 1 in 200 appendices removed for acute appendicitis is found to have a carcinoid. Generally they require no treatment unless there has been evidence of metastatic spread to lymph nodes or omentum, or if a tumor is greater than 2 cm in size. These tumors contain argentaffin cells derived from Kulchitsky's cells of the small intestine. These secretory cells are thought to have endocrine functions, as the tumors may produce symptoms referred to as the carcinoid syndrome, characterized by elevated levels of serotonin in the blood and urine. [286,287 and 288] With the carcinoid syndrome, affected patients have periodic flushing, diarrhea, bronchoconstriction, peripheral vasomotor symptoms, and cyanosis. [286,287 and 288] These symptoms are attributed to circulating 5-hydroxytryptamine (serotonin) and histamine. Urinary levels of 5-hydroxyindoleacetic acid are elevated. Treatment is surgical if possible. The appropriate diagnostic evaluation depends on the tumor sites. Octreotide scans and measurement of 24-hour urinary excretion of 5-hydroxyindoleacetic acid may confirm the diagnosis.

If the tumor is malignant and has spread in a manner similar to colorectal carcinoma, chemotherapy may be beneficial. [289,291] Patients with intra-abdominal and pulmonary metastases may be treated with combinations of chemotherapy that include doxorubicin.

Regional ileocolectomy has been advocated for extension of carcinoids to the meso-appendix and serosal fat with indeterminate or inadequate surgical resection margins. [287]

Although malignant carcinoid is rare, it may present with massive hepatic enlargement and metastases in association with the carcinoid syndrome. [290,291] Rarely is the carcinoid tumor considered at the time of initial presentation, which may result in diagnostic delay.

Patients with metastatic disease at diagnosis fair poorly in that curative treatment for these individuals is undefined. Octreotide and its analogs may palliate the symptoms, yet both the long-acting and the short-acting somatostatin derivatives may not completely control the symptoms.

Bronchial carcinoids may present with evidence of obstructive bronchial disease or pneumonia. Most bronchial carcinoids are not malignant, but they may require lobectomy for resection of tumor. [289]

## CHORDOMA

This rare neoplasm arises from notochordal remnants in the midline of the neuraxis and involves adjacent bone. The coccyx, sacrum, and base of the skull are most often involved. [292,293,294,295 and 296] Differential diagnosis includes myxoid chondrosarcoma and metastatic carcinoma. This tumor is generally fatal because of a high rate of local recurrence. The most common complaints for the sacrococcygeal tumors include pain, constipation, and sensory loss. These individuals present with a large presacral mass.

Surgery is the most important of the modalities to be used initially, but radiation therapy with doses of 5,500 to 7,000 cGy may prolong local control. Little information is available regarding appropriate chemotherapy. With dissemination there may be widespread disease in lung, liver, and bone.

## CANCER OF UNKNOWN PRIMARY SITES

From 1973 to 1987, more than 1 million cases of cancer diagnosed in residents of Surveillance, Epidemiology, and End Results program areas, and approximately 2% were designated as cancers of unknown primary sites. [297] The most frequent diagnosis was adenocarcinoma. [298,299 and 300] There has, however, been a decline in the numbers of cancers assigned to unknown primary sites. Other more common types of tumors in this category would include melanomas or embryonal tumors such as rhabdomyosarcoma and neuroblastoma. [301,302,303,304 and 305] The patients generally present with lymph node metastases or generalized disease. Comparative studies have been made for melanoma patients who presented with an unknown primary site in comparison to patients with known primary sites. [305] Molecular and cytogenetic studies may be useful in the diagnosis and prognosis of these tumors. [306] After completion of the diagnostic pathological studies and imaging studies, treatment should be based on the pathology regardless of knowledge of the primary site. [302,303,304,305,306,307 and 308] For these patients, prompt initiation of treatment may lead to clinical response and possible cures.

## CANCERS OF THE SKIN

More than 700,000 cases of basal cell and squamous cell carcinoma occur annually in the United States, accounting for nearly 40% of all adult cancers. [309] Basal cell carcinoma is the most common form of skin cancer in adults and accounts for 75% of the cases. The mortality rate for these two cancers is 2,100 per year. The incidence of melanoma, a less frequent but more invasive and fatal skin neoplasm, has drastically increased over the past 60 years. In 1997, over 40,000 cases of melanoma were diagnosed in the United States, and 7,200 of those patients died from their disease. [309] In children younger than 20 years, melanoma is the second most common epithelial cancer, accounting for 30.9% of all cases. [310]

Basal cell and squamous cell carcinomas may be curable with surgery and radiation therapy and will not be discussed at length in this chapter. Small lesions may respond to a single dose of 2,200 cGy, but larger lesions may require fractionated doses up to a total of 6,000 cGy.

### Epidemiology

Increased total sun exposure has been implicated as an important factor underlying the pathogenesis and increased incidence of skin cancer. The incidence of melanoma and nonmelanoma skin cancers increases exponentially with age, and its incidence is also highly dependent on regional differences. [311] Ultraviolet B wavelengths (290 to 320 nm) are responsible for formation of cyclobutane pyrimidine dimers and pyrimidine photoproducts whose altered repair leads to mutations and the formation of squamous cell carcinomas in mice. Ultraviolet A wavelengths (320 to 400 nm) are more abundant in sunlight and also cause oxidative DNA damage and immunosuppression which, in certain animals, can cause melanoma. Melanocytes, unlike keratinocytes, have a limited proliferative capacity. Cumulative sun exposure has been linked to the development of basal and squamous cell carcinomas, whereas intermittent high-dose exposures (sunburns), particularly during childhood, have been correlated with an increased risk of melanoma. [309,312] The risk for basal and squamous cell carcinomas increases in light-skinned individuals and in those who freckle easily, and is related to the amount of solar exposure received.

Experimental skin cancer was produced in mice by exposure to ultraviolet light in 1928. Epidemiologic data from various global latitudes have shown an increase in squamous cell and basal cell carcinomas in white populations closer to the equator. [314] Certain phenotypes are associated with greater susceptibility to skin cancer within any geographic area; these persons tend to have poor tanning ability with easy sunburning and often have blue eyes, light hair, and fair skin. There is no gender difference in the incidence of any types of skin cancer in the pediatric age groups.

There are two major effects of ultraviolet radiation on the skin that may be responsible for its carcinogenic effects. These are photochemical alteration of DNA and alterations in immunity. As predicted, xeroderma pigmentosum cells in culture fail to repair ultraviolet damage to DNA because the thymine dimers are not excised. [316] Chemical carcinogens may also be associated with the development of basal cell and squamous cell carcinomas. This was first described by Pott, who noticed the

high incidence of carcinoma of the scrotal and penile skin in chimney sweeps in England. [310]

Ionizing radiation is also associated with carcinogenesis of the skin. [312] Early reports involved workers using early x-ray machines. In children, radiation-associated basal cell carcinomas generally have occurred in patients treated for acute lymphocytic leukemia with central nervous system prophylaxis or for Hodgkin's disease with mantle irradiation techniques.

**Melanoma**

Childhood melanoma is rare, accounting for 1% to 2% of all pediatric malignancies. Of the estimated 46,000 new cases of melanoma diagnosed in the United States each year, only 1% to 4% occur in patients younger than 20 years and 0.4% among those younger than 14 years. Melanoma is second only to thyroid cancer as the most common carcinoma of children.[309,313,314] At our center, melanoma accounted for 1% of all solid tumors and 0.5% of all cancers among children who were seen or treated over a 33-year period. Contributing factors to the development of melanoma which appear to be restricted to the pediatric population include the following:

1. Congenital melanomas may develop *in utero* in the absence of melanoma in the mother. They can arise in a giant melanocytic nevus or may develop de novo. Cutaneous melanoma is the most common transplacentally transmitted neoplasm and carries a poor prognosis.[314,315]
2. Giant congenital melanocytic nevi affect fewer than 1 in 20,000 newborns and are precursor lesions of melanoma ( Fig. 38-4).[316,317] The life time risk of developing melanoma has been estimated to be 4.6% to 8.5%.[320] Over one-half of all giant congenital melanocytic nevi–associated melanomas develop during the first decade of life.[314,316,317]

**FIGURE 38-4.** Giant pigmented nevus in a 4-year-old child. Notice the “bathing trunk” appearance of the nevus and numerous satellites on all portions of the body. Lesions have faded with the aging of this patient.

3. Xeroderma pigmentosum is a rare (1:500,000) inherited excisional DNA repair disorder characterized by photosensitivity, a greater than1,000-fold increased risk of skin cancer in patients younger than 20 years, and in some cases, neurologic manifestations. [312,318] Malignant skin neoplasms develop in 70% of patients. The median age at diagnosis is 8 years. Fifty-seven percent develop squamous and basal cell carcinomas, and up to 22% develop melanoma. [318]
4. Immunosuppression: Children with immunodeficiencies have a three- to sixfold increased risk of developing melanoma, and those with Hodgkin's disease have an eightfold increased risk.[315] Melanoma after renal transplantation and immunosuppressive therapy has also been described. [319] A significant increase in nevus counts (mean, 66 per patient) has also been observed in children after completion of maintenance chemotherapy for acute lymphoblastic leukemia or Hodgkin's disease.[320]
5. Neurocutaneous melanosis is a rare syndrome characterized by large or multiple congenital nevi associated with meningeal melanosis or melanoma. The majority of patients have giant pigmented lesions along the posterior midline or in the head and neck region. In symptomatic patients, neurologic manifestations are evident by age 2 years and include hydrocephalus, seizures, papilledema, headaches, and mental retardation. Prognosis is poor, with only 18% of patients expected to survive. Leptomeningeal melanoma has been reported in 64% of cases and only 18% of affected children survived. [321] An asymptomatic form of neurocutaneous melanosis characterized by abnormal brain MRI T1 shortening in the amygdala, cerebellum, or pons has been recently described. All patients had giant congenital melanocytic nevi involving the skin underlying the dorsal spine or scalp, and 23% had MRI abnormalities. Yet, only 1 of 46 patients evaluated developed neurological symptoms.[322]
6. Mole phenotype: It is estimated that 44% of melanomas in people younger than 30 years arise in a small nevus that was present at birth or during early childhood.[323,324] The risk of developing melanoma in patients with atypical moles and a negative family history is approximately 5%; however, the risk is significantly higher for patients who are members of melanoma-prone families (two members diagnosed with melanoma).[324] Interestingly, 10% of melanomas occurred in patients younger than 20 years, and the median age at diagnosis of melanoma decreased by 11 to 16 years per generation. [324,325] More recently, germline mutations of the *p16* gene, which localizes to region 9p21, have been implicated in the development of melanoma in families prone to this malignancy.[326]

The majority of published reports of pediatric melanoma are single-institution studies, in which diagnostic criteria, staging systems, and therapy regimens have varied widely. Furthermore, too few patients with advanced-stage disease have been reported, so definitive conclusions regarding therapy recommendations cannot be made. The available literature suggests, however, that the natural history and response to therapy of pediatric melanoma is stage dependent, similar to that in adults. Among 588 pediatric patients reported in ten separate series, the sex distribution was similar among males and females. Most patients were white, and at least 80% of cases presented during the second decade of life. Primary tumors occurred most frequently in the extremities, followed by those in the trunk and the head and neck region. Metastatic disease at presentation was rare, with only 11 cases reported. Associated conditions, such as preexisting mole, pigmented lesion, or giant congenital melanocytic nevus were reported in 115 of the 588 patients. Xeroderma pigmentosum, familial and congenital melanoma, and dysplastic nevus syndrome were seen infrequently.[327]

The more common types of melanomas are characterized by indolent, peripheral enlargement of relatively flat, complex colored, primary lesions. The indolent growth phase (i.e., radial growth phase) may take place over several years, during which the tumor has little competence to metastasize. The ability to metastasize is associated with penetration of the tumor into deeper cutaneous tissues (i.e., vertical growth phase). Common clinical manifestations include increased size of a preexisting mole, bleeding, itching, color change, palpable subcutaneous mass, and palpable lymphadenopathy. [328]

The patterns of spread of melanoma in pediatric patients are in all ways similar to those of adults with the disease, with satellitosis and regional lymph nodes becoming involved before involvement of abdominal viscera, lungs, bone, or brain ( Fig. 38-5). Survival of patients with pediatric melanoma is similar to that of adults and is stage dependent. Thick melanomas, nodal involvement, or metastatic disease at diagnosis predict a poor clinical outcome. In one series, 74% of recurrences were seen in children whose lesions were greater than or equal to 1.5 mm. Other pediatric series showed uncharacteristically high incidences of late recurrences and nodal metastases. In a study from our institution that spanned 21 years, all children with lesions smaller than 1.5 mm survived, but only 10 of 22 patients with 1.5-mm or larger lesions survived. None of the patients whose melanomas were characterized by a Breslow's thickness of less than 1.5 mm developed nodal disease; in contrast, 63% of those whose tumors were at least 1.5 mm in thickness developed nodal metastases.[329]

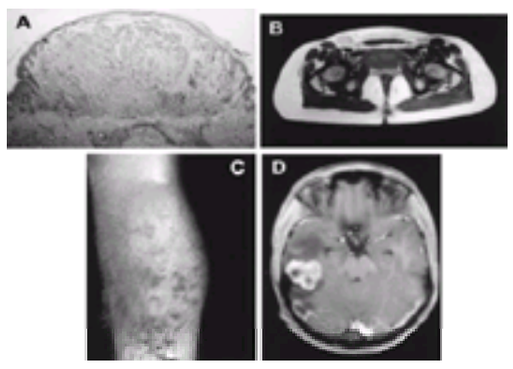

**FIGURE 38-5.** Various clinical and pathologic aspects of pediatric melanoma. **A:** Nodular melanoma in an adolescent. Breslow's thickness at diagnosis was 2.7 mm. **B:** Axial T1 pelvic magnetic resonance imaging (MRI) showing T1 hyperintense right inguinal nodal metastases from malignant melanoma in a 13-year-old girl. **C:** Sixteen-year-old boy who, at the time of recurrence of melanoma, developed multiple in-transit metastases, a phenomenon believed to represent lymphatic dissemination of the disease. **D:** Axial T1 MRI of brain with contrast showing a large enhancing right temporal lobe metastases from melanoma with surrounding edema in a 13-year-old girl.

### *Staging*

Table 38-5 depicts the current AJCC staging system for melanoma of the skin, published in January 2000. This new classification stratifies patients according to the thickness of the primary tumor, presence or absence of nodal metastases, ulceration, or metastatic disease. [330] Incorporation of this system in future pediatric melanoma trials will allow uniform reporting of cases and should facilitate interpretation of results when analyzing and comparing single-institution reports. Because comprehensive guidelines for staging children with newly diagnosed melanoma have not been established, we use the National Comprehensive Cancer Network guidelines at our center to stage the majority of pediatric patients with melanoma. In our experience, however, routine diagnostic imaging of asymptomatic patients can identify metastases in one-fourth of pediatric patients. [331] We therefore recommend that children with malignant melanoma who present with localized thick lesions (greater than 4 mm) and those with regional nodal disease undergo chest CT and MRI of the locoregional nodal draining basin to define the primary tumor burden. Because bone scintigraphy and head MRI do not efficiently detect clinically silent metastases, we do not recommend their routine use in asymptomatic patients with thick lesions.

**TABLE 38-5. CURRENT AMERICAN JOINT COMMITTEE ON CANCER STAGING SYSTEM FOR CUTANEOUS MELANOMA**

### *Treatment*

#### Primary disease

As in adults, early detection and early surgical removal of primary melanoma remain the most effective treatment for children. Suspicious skin lesions (i.e., those with irregular borders, pigmentation, or texture) should be surgically removed and submitted for histopathologic examination. If melanoma is documented, the invasiveness and depth of the lesion should be determined. Lesions less than or equal to 1 mm in thickness should be resected with a 1-cm margin; those 1 to 4 mm are excised with a 2-cm margin. To minimize the risk of local recurrence, we use margins of at least 2 cm for lesions greater than 4 mm in thickness. [332]

The routine use of elective lymph node dissection has been largely discontinued given the results of the most recent adult Intergroup Surgical Melanoma trial in which only a subset of patients, (lesions 1 to 2 mm thick, age younger than 60 years, no ulceration) benefited from this procedure. [333] In addition, the advent of intraoperative lymphatic mapping with selective lymphadenectomy has proven highly effective and sensitive in identifying occult nodal disease. This procedure is routinely used at our center to stage all patients with melanoma who present with lesions greater than or equal to 1mm in thickness.

**Adjuvant Therapy**. The role of adjuvant therapy in pediatric melanoma has not been prospectively studied. An Eastern Cooperative Oncology Group trial documented the efficacy of adjuvant interferon alpha-2b in prolonging relapse-free and overall survival in adult patients with high-risk, completely resected melanoma.[334] However, a subsequent trial has failed to demonstrate a significant survival advantage for patients who received this treatment modality. [335] Thus the role of this treatment modality in children with completely resected nodal disease and with lesions greater than 4 mm remains uncertain and warrants future study.

#### Disseminated Disease

**Chemotherapy**. Dacarbazine has been used before surgery with encouraging results in four children with melanoma. [336] Hayes and Green[337] reported responses to vincristine, dactinomycin, and cyclophosphamide in seven of nine children with advanced-stage melanoma. We studied a regimen that incorporated alternating courses of cisplatin and etoposide to the vincristine/dactinomycin/cyclophosphamide with concurrent interferon alfa-29. Three of four children with advanced stage disease (IIb–IV) who were treated with surgery and chemotherapy remain disease-free, whereas only one of four children with unresected disease survived.

**Interleukin 2**. IL-2 has modest activity in adult melanoma.[338] Two pediatric trials have used IL-2 in the treatment of refractory solid tumors, but no significant responses were observed. Only one response (in a child with renal cell carcinoma) was observed. Currently, there is insufficient information documenting the clinical activity of IL-2 in pediatric melanoma.

**Other Therapies**. Davidoff and colleagues[339] treated children whose melanomas were thicker than 1 mm or had aggressive features (e.g., ulceration or head and neck location) with subcutaneous injections of $2.5 \times 10^7$ irradiated allogeneic cultured melanoma cells. The response, tolerance, or side effects to this treatment were not described in this report.

**Biochemotherapy**. We are currently using a concurrent biochemotherapy regimen devised by Legha and colleagues [340] for patients with metastatic disease. This regimen incorporates cisplatin, dacarbazine, vinblastine, IL-2, and interferon. In adults, 64% of patients achieved an objective response and nearly half of those who achieved a complete response were long-term survivors.

Finally, it is obvious that melanoma may be a preventable disease. With a decrease in the enthusiasm for sun exposure by young persons and with the judicious use of sunscreen products, a significant reduction in the frequency of melanoma and nonmelanoma skin cancer may be expected.

#### Nevoid Basal Cell Carcinoma Syndrome (Gorlin's Syndrome)

Gorlin's syndrome is an autosomal dominant disorder characterized by developmental abnormalities including rib and craniofacial anomalies, odontogenic keratocysts of jaws, epidermal skin cysts, and palmar or plantar pits (Fig. 38-6). In addition, these patients are prone to the development of a variety of tumors, including fibromas of the ovaries and heart.[341] Approximately 5% of patients with Gorlin's syndrome develop medulloblastomas. It is estimated that up to 10% of patients with medulloblastoma have Gorlin's syndrome. The gene found to be mutated in Gorlin's syndrome, *patched,* is located at 9q22 and influences development by regulating transcription of several genes including *gli,* members of the tumor growth factor b, and Wnt family of transcription factors.[342]

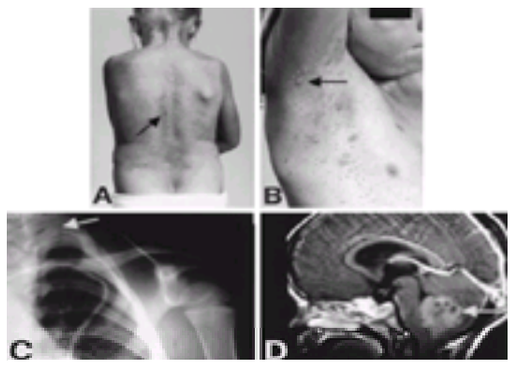


**FIGURE 38-6.** The clinical spectrum of Gorlin's syndrome. **A:** Multiple basal cell carcinomas (*arrow*) along the craniospinal radiation therapy field in a patient with Gorlin's syndrome who was treated for medulloblastoma. **B:** Multiple epidermal cysts and basal cell carcinomas (*arrow*) in the right axillary region. **C:** Posteroanterior radiograph of chest shows fusion of the first and second ribs (*arrow*). **D:** Sagittal T1 brain magnetic resonance imaging demonstrating enhancing fourth ventricle medulloblastoma (*arrow*).

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39

# ONCOLOGIC EMERGENCIES

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BEVERLY J. LANGE



## INTRODUCTION

Emergencies can appear at any time during a child's course of care for cancer. Some emergencies are the initial manifestation of cancer or develop as the diagnosis is being made; others arise as a consequence of therapy or at the time of tumor recurrence. Oncologic emergencies can arise from metabolic perturbations that result from the malignancy or therapy to treat the malignancy, from space-occupying lesions that obstruct vital organs, or from hematologic abnormalities.

This chapter addresses pediatric oncologic emergencies by system—thoracic, abdominal, genitourinary, metabolic, and central nervous system—and summarizes the management of cardiovascular collapse and shock, the end result of uncompensated emergent situations. Chapter 40 reviews management of emergencies associated with cytopenias and hemostasis and the use of blood component therapy. Infectious complications are addressed in Chapter 41 and principles of pain management in Chapter 43.

## CARDIOTHORACIC EMERGENCIES

Respiratory distress is a common presenting symptom of intrathoracic malignancies and is often the sole symptom of a cardiothoracic emergency occurring at any time. The causes of respiratory distress can be classified by their location in the thorax. In the anterior mediastinum, superior vena cava syndrome (SVCS) and superior mediastinal syndrome (SMS) are the major emergencies. Intracardiac masses, cardiac tamponade, and myocardiopathy or myocarditis are the emergent events in the middle mediastinum. Mass lesions in the posterior mediastinum do not cause respiratory symptoms but may cause cord compression (see the section on Neurologic Emergencies). Intrapulmonary processes include infiltrates, pneumothoraces, masses, and fibrosis; intrapleural processes, masses and effusions; and cardiac processes, masses, effusions, fibrosis, and failure.

### Superior Vena Cava Syndrome and Superior Mediastinal Syndrome

*SVCS* refers to the signs and symptoms resulting from compression, obstruction, or thrombosis of the superior vena cava. The term *SMS* is used when tracheal compression also occurs. In children with mediastinal masses, tracheal compression and respiratory embarrassment usually coexist with SVCS and, therefore, *SVCS* and *SMS* often are used synonymously.

#### *Etiology*

SVCS in children is rare. In 1983, Issa et al.[1] summarized the frequency and causes of SVCS in 150 children and adolescents. Although most were thrombotic complications of cardiovascular surgery for congenital heart disease, the most common primary cause of SVCS in children was cancer.[1,2] Mediastinal granulomas, infections such as histoplasmosis, or venous thrombosis from central venous lines (CVLs) may cause SVCS and SMS that are clinically indistinguishable from cancer.[3,4]

Table 39-1 lists the frequency of SVCS and SMS among 3,721 children with cancer treated at St. Jude clinically indistinguishable from cancer.'s Research Hospital.[2] Almost 70% of patients with non-Hodgkin's lymphoma (NHL) and 30% with Hodgkin's disease (HD) presented with mediastinal masses. Patients with neuroblastoma, germ cell tumors, sarcomas, and acute lymphoblastic leukemia (ALL) also were found to have mediastinal masses at diagnosis. In up to 75% of children with mediastinal masses, some respiratory compromise was evident.[5] SVCS was most commonly associated with NHL, followed closely by ALL, but also occurred with other tumors.[2]

| Diagnosis | No. of patients | Mediastinal mass (%) | SVCS with mediastinal mass (%) |
|---|---|---|---|
| Acute lymphoblastic leukemia | 1,464 | 130 (8.4) | 6 (4.6) |
| Acute nonlymphocytic leukemia | 392 | 9 (2.3) | 0 |
| Hodgkin's disease | 333 | 102 (30.6) | 2 (2.0) |
| Non-Hodgkin's lymphoma | 330 | 230 (69.7) | 8 (3.4) |
| Neuroblastoma | 332 | 69 (20.8) | 3 (4.3) |
| Germ cell tumors | 114 | 10 (8.8) | 2 (20.0) |
| Sarcomas | 696 | 26 (3.7) | 3 (11.0) |

From Ingram L, River G, Shapiro DDN. Superior vena cava syndrome associated with childhood malignancy. Analysis of 24 cases. Med Pediatr Oncol 1990;18:476, with permission.

**TABLE 39-1. INCIDENCE OF MEDIASTINAL MASS AND SUPERIOR VENA CAVA SYNDROME (SVCS) AT ST. JUDE CHILDREN'S RESEARCH HOSPITAL BETWEEN 1973 AND 1988**

### *Pathophysiology*

The SVC is a thin-walled vessel with low intraluminal pressure. It is surrounded by lymph nodes and the thymus. Tumor or infection in the mediastinal nodes or thymus can compress the SVC, causing venous stasis. The adjacent pericardium and coronary or collateral vessels fill with tumor or clot. The trachea and right main stem bronchus in young children are more compliant and compressible than in the adult. Symptoms of compression are especially pronounced in infants, as their tracheas and bronchi have small intraluminal diameters. Compression, clotting, and edema combine to minimize tracheal airflow and reduce venous return from the head, neck, and upper thorax, causing the signs and symptoms of both SVCS and SMS.

### *Evaluation*

The most common symptoms of SVCS and SMS in children are dyspnea, cough, dysphagia, orthopnea, and hoarseness (Table 39-2).[2,6] Anxiety, confusion, lethargy, headache, distorted vision, and syncope indicate carbon dioxide retention and central venous stasis. Symptoms typically are aggravated when the patient is supine [as for an abdominal examination or computed tomography (CT) scan] or is placed in a fetal position (as for lumbar puncture). Characteristic physical findings include edema, plethora, and cyanosis of the face, neck, and upper extremities; cervical and thoracic venous distention; conjunctival suffusion and edema; and wheezing or stridor.[2,6] Signs of pleural and pericardial effusions may coexist. In adults, onset of SVCS caused by a malignant tumor, usually lung cancer, is insidious. In children and adolescents, the symptoms often progress rapidly over days.

| Finding | No. (%) |
|---|---|
| Coughidyspnea | 11 (68) |
| Dysphagiaorthopnea | 10 (63) |
| Wheezing | 5 (31) |
| Hoarseness | 3 (19) |
| Facial edema | 2 (12) |
| Chest pain | 1 (6) |
| Pleural effusion | 8 (50) |
| Pericardial effusion | 3 (19) |

From Ingram L, River G, Shapiro DDN. Superior vena cava syndrome associated with childhood malignancy. Analysis of 24 cases. Med Pediatr Oncol 1990;18:476, with permission.

**TABLE 39-2. SYMPTOMS AND PHYSICAL FINDINGS IN PATIENTS WITH SUPERIOR VENA CAVA SYNDROME AT INITIAL PRESENTATION**

A child with some or all of these signs and symptoms must undergo chest radiography. Figure 39-1A shows mediastinal widening on a posteroanterior film. Figure 39-1B shows the mass to be in the anterior mediastinum as it is opacifying the retrosternal space. Most children with SVCS will have a mass in the anterosuperior mediastinum. Pleural and pericardial effusions are more common in NHL than in HD or other malignancies.[6] A chest radiograph may show tracheal deviation. Patients with tumors larger than 45% of the transthoracic diameter are more likely to be symptomatic than are those with ratios of less than 30% (i.e., smaller tumors).[6,7] CT scans can delineate the distortions of normal anatomy and more accurately assess the extent of tracheal compression. CT can be performed with the patient in the prone position if the supine position aggravates the respiratory distress. If the cause of symptoms is suspected to be a thromboembolism or pericardial effusion, an echocardiogram should be obtained. Pulmonary function tests and volume flow loop assess pulmonary reserve and resilience.

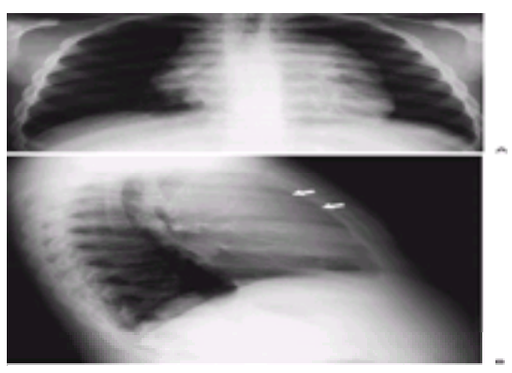

**FIGURE 39-1.** Posteroanterior **(A)** and lateral **(B)** views of the chest show anterior mediastinal widening in a 16-year-old boy with Hodgkin's disease. Note soft tissue opacifying the retrosternal space (*arrows*) on the lateral view **(B)**. (Courtesy of James S. Meyer, M.D., Children's Hospital of Philadelphia.)

When cancer is the probable cause of SMS, it is desirable to obtain a tissue specimen for diagnosis. However, a child with SVCS or SMS may not tolerate anesthesia for a mediastinal biopsy.[8] During general anesthesia, respiratory muscle tone decreases, abdominal muscle tone increases, the caudal movement of the diaphragm disappears, bronchial smooth muscle relaxes, and lung volume diminishes.[8] These changes aggravate the effects of extrinsic compression of the vena cava. Tracheal intubation may be extremely difficult, and some patients will not be able to be extubated until the tumor bulk has been reduced. Conscious sedation or anti-anxiolytics may also be contraindicated, as they decrease respiratory drive and dilate peripheral vessels, thereby reducing venous return.[8,9]

Several recent reports suggest a stepwise approach to diagnosis.[8,9] An algorithm appropriate for pediatric patients is shown in Figure 39-2. Diagnosis should be made in the most expeditious and least invasive manner possible. A differential diagnosis must be generated on the basis of history, physical examination, and simple laboratory tests. A complete blood cell count (CBC) showing cytopenias or blast cells may indicate leukemia, whereas a leukocytosis with an extreme leftward shift may favor diagnosis of a bacterial infection, which rarely causes SVCS or SMS. Marrow aspiration performed under local anesthesia with the patient upright or prone may reveal leukemia or lymphoma. In a patient with a central venous catheter, echocardiography may demonstrate a thrombus. Pleurocentesis or pericardiocentesis can offer immediate relief and provide diagnostic material. If an enlarged peripheral lymph node is present, node biopsy is faster and less invasive than is a

mediastinal biopsy. Patients who cannot tolerate these procedures will not tolerate general anesthesia or even conscious sedation.

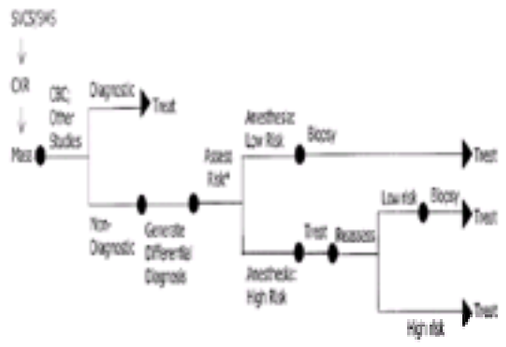


**FIGURE 39-2.** Assessment and management of a child with respiratory distress, superior mediastinal syndrome (SMS), or superior vena cava syndrome (SVCS) and an anterior mediastinal mass. Initial assessment for anesthetic risk may include computed tomography of the chest, echocardiography, pulmonary function tests, and flow volume loop. If the patient cannot tolerate these studies, or if the studies indicate severely compromised cardiopulmonary reserve, the patient is a high anesthetic risk. CBC, complete blood cell count; CXR, chest radiograph.

***Therapy***

In a child who presents with SVCS or SMS as an initial symptom of malignancy, establishing a tissue diagnosis may be impossible, and starting empiric therapy may be medically necessary. Traditionally, emergency therapy consisted of irradiation, as most lymphomas are exquisitely radiosensitive. However, an increasingly popular trend is to initiate emergent systemic chemotherapy. No established standards exist.

Between 1988 and 1994, 10% of pediatric oncology patients at the Children's Hospital of Philadelphia referred for emergent radiotherapy had respiratory difficulties associated with SMS; 83% responded to the radiotherapy.[10] In NHL, radiotherapy is effective, and improvement can occur within 12 hours.[10] However, some lymphomas are so radioresponsive that doses as low as 200 cGy to a circumscribed area of the tumor can cause rapid dissolution of the mass and regional lymph nodes, making subsequent tissue diagnosis impossible. Loeffler et al.[11] reported that of 19 patients with mediastinal masses, emergency prebiopsy irradiation rendered the histologic specimen uninterpretable in 8. Seven of the eight patients were treated empirically for HD or NHL. Four had no tumor recurrence, three had recurrence with the disease they were assumed to have had, and the one untreated patient developed recurrent seminoma. The authors point out that patient management was not altered by prebiopsy therapy or by continued empiric therapy.[11]

A second problem is respiratory deterioration, presumably from tracheal swelling after irradiation. The phenomenon of postirradiation deterioration is limited to children and adolescents, perhaps because of the greater compressibility of their respiratory structures and the inability of their relatively narrow lumina to accommodate postirradiation edema. Increasingly, radiation oncologists are using highly focused radiation portals, such as a small field centered on the trachea only, to circumvent the problem of swelling of small airways.[10,12] Another option consists of using small, bilateral, opposing fields encompassing the trachea, SVC, and proximal right auricle. The daily dose is governed by the presumed radiosensitivity of the tumor, with 100 to 200 cGy given twice daily for radioresponsive tumors such as lymphoblastic lymphoma or leukemia. A dose of intravenous methylprednisolone (1 mg per kg) followed by prednisone at 40 mg per $m^2$ daily, divided into two or three doses, may prevent worsening of the respiratory distress but may confound the ability to make a diagnosis.

Chemotherapy, including steroids or cyclophosphamide or both, is a reasonable alternative to irradiation. Unfortunately, chemotherapy can also confound the diagnosis, as the histologic picture may be rendered uninterpretable within 48 hours.[7,9] Failure to persist with treatment for the presumed leukemia or lymphoma, even if a histologic diagnosis is not made, may allow the disease to progress to a more advanced stage.[9,11] Chemotherapy rather than irradiation is indicated in ALL accompanied by a high leukocyte count and a mediastinal mass causing SMS or SVCS. Chemotherapy treats both life-threatening problems: SVCS and hyperleukocytosis. However, if renal failure is present or impending, irradiation is the treatment of choice for the SVCS or SMS, to avoid tumor lysis syndrome (TLS).

Progressive symptoms may be caused by a less responsive neoplasm such as a teratoma, large-cell lymphoma, neuroblastoma, germ cell tumor, or benign tumor, making surgical resection inevitable. Surgery should be performed with the patient in the semi-Fowler's position, with advance preparations made for a change of position to lateral or prone, and with cardiopulmonary bypass facilities and a rigid bronchoscope on standby.[8]

If signs or symptoms of SVCS develop in a child with a CVL, one must be concerned about an extensive thromboembolism. This situation is usually a subacute emergency. If there are no contraindications, the catheter should be kept in place initially for infusion of thrombolytic therapy. Recent recommendations from the Children's Thrombophilia Network for systemic thrombolytic therapy are to use tissue plasminogen activator (TPA) at a dose of 0.1 to 0.6 mg per kg per hour for 6 hours.[13] Fibrinogen, fibrin split products, prothrombin time, and activated partial thromboplastin time should be monitored closely. Systemic heparin therapy should be begun either during or immediately after thrombolytic therapy with a loading dose of 75 units per kilogram, followed by a maintenance dose of 20 units per kilogram for children older than 1 year. The infusion rate should be modified to achieve a partial thromboplastin time of 60 to 85 seconds.[13] For less symptomatic CVL-associated thromboembolisms, heparin alone at the aforementioned doses may suffice and has fewer systemic side effects. Continued anticoagulation for 3 to 6 months with oral Coumadin or subcutaneous low-molecular-weight heparin is recommended.[14]

## Pleural and Pericardial Effusions

***Etiology and Pathogenesis***

An effusion represents the escape of fluid into a potential space. Effusions are exudates or transudates. Exudates have protein concentrations in excess of 2.5 g per dL, a specific gravity greater than 1.015, a pH of less than 7.3, and a high white blood cell (WBC) count. Exudates are caused by either the primary malignancy or infection. In contrast, transudates result from a sympathetic response to tumor in the chest or abdomen, fluid overload, heart failure, or hypoproteinemia. Protein concentration, specific gravity, pH, and cell counts are low in transudates. Chylous effusions can occur from obstructed lymphatic channels.[15]

***Evaluation***

Symptoms of both pericardial and pleural effusions include dyspnea, orthopnea, chest pain, and cough.[16] Small, clinically silent pleural and pericardial effusions often are detected incidentally on radiographs and echocardiograms. Asymptomatic effusions can be monitored without intervention. However, a large accumulation of pericardial fluid can cause cardiac tamponade and rapid decompensation. Therefore, emergent thoracentesis or pericardiocentesis is indicated to relieve respiratory or cardiac distress, obtain diagnostic fluid, or eliminate a potential reservoir for such drugs as methotrexate. Fluid should be sent for measurement of cell count, protein content, cytology, Gram stain, culture, and assays of appropriate immunologic and biologic markers.

***Therapy***

In a child with untreated cancer and respiratory distress, a single removal of fluid often is sufficient, as the fluid usually does not reaccumulate once therapy is under way. In contrast, children with advanced malignant disease may develop recurrent effusions that compromise their duration and quality of life. Palliative measures include repeated centeses, placement of a percutaneous catheter, or instillation of a sclerosing agent into pleural or pericardial cavities to cause irritation and adhesion of the potential space. Owing to the resultant scarring, fibrosis, and adhesions, the instillation of a sclerosing agent can make difficult the interpretation of images obtained subsequently. In pediatrics, the use of sclerosing agents usually is reserved for palliation of refractory pleural disease.

Tetracycline is the most widely used sclerosing agent and has the fewest side effects. The effusion is drained, and 500 to 1,000 mg of tetracycline in 20 mL of 0.9% saline is instilled into the cavity.[17,18] The procedure may need to be repeated daily for several days. In one study, tetracycline prevented reaccumulation of pericardial

fluid in 30 of 33 patients.[18] A review of 1,168 patients showed a 93% success rate with talc.[16] Talc itself is inexpensive, but its instillation requires thoracoscopy and, usually, general anesthesia. In 8 of 11 children with malignant solid tumors, intracavitary cisplatin, 100 to 200 mg per $m^2$, with 16 to 52 g of thiosulfate per $m^2$ (or 300 mL per $m^2$ in an infant) in 2 L of warmed normal saline or 50 to 210 mg per $m^2$ without thiosulfate rescue eliminated the intracavitary disease.[19] Intracavitary therapy achieved drug concentrations 40-fold higher than systemic therapy.[19] If sclerosing agents fail, surgical pleurectomy or pericardiectomy may be necessary. However, surgery entails substantial morbidity and potential mortality for patients with resistant metastatic disease.

## Cardiac Tamponade

### *Etiology and Pathogenesis*

Cardiac tamponade occurs when the left ventricle fails to maintain output because of compression by pericardial fluid or leukemic infiltration, inflammation or infection of the pericardium, constrictive fibrosis from previous irradiation, or occlusion from tumors of the cardiac muscle or endocardium.[20,21] Infectious pericarditis or myocarditis probably is the most common cause of tamponade in immunocompromised children with cancer. Intracardiac masses such as marantic vegetations or clots also can cause cardiac tamponade. A Wilms' tumor thrombus can extend from the renal vein through the tricuspid valve to fill the right cardiac chambers. Although leukemia may cause pericardial effusions at diagnosis, cardiac tamponade is rarely the presenting symptom of an undiagnosed malignancy.

### *Evaluation*

Gradual accumulation of fluid allows the pericardium to accommodate a large volume, but rapid accumulation of several hundred milliliters can cause sudden decompensation. Symptoms of impending tamponade resemble those of congestive heart failure: cough, chest pain, dyspnea, hiccups, and abdominal pain. Signs include tachycardia, cyanosis, hypotension, and a pulsus paradoxus of greater than 10 mm.[22] Tamponade must be differentiated from congestive heart failure, infectious pericarditis or myocarditis, and therapy-induced cardiomyopathy. Constrictive pericarditis may cause friction rubs, diastolic murmurs, and atrial arrhythmias.

Radiographs of large pericardial effusions often show a typical “waterbag” cardiac shadow on an anteroposterior view and an abnormal space between the pericardial fat and pericardium on the lateral view. Pleural effusions may also be noted. The echocardiogram may show low-voltage QRS complexes, flattened or inverted T waves, and electrical atrial and ventricular alternans.[22] Echocardiography of the posterior wall displays two echoes, one from the cardiac muscle and one from the pericardium. An echocardiogram may also reveal a thickening of the pericardium consistent with pericarditis or pericardial tumor.

### *Therapy*

Supportive care for malignant pericardial effusion and constrictive pericarditis consists of hydration, oxygen, and patient positioning to maximize cardiac output. Diuretics are contraindicated as hypovolemia decreases stroke volume.[22] Definitive treatment of tamponade caused by an effusion is immediate removal of fluid under echocardiographic guidance. Of nine pediatric patients treated by percutaneous catheter drainage at the Memorial Sloan-Kettering Cancer Center, all experienced symptomatic relief, and eight showed complete echocardiographic resolution.[20] Alternative but more invasive procedures, such as subxyphoid pericardiotomy or pericardial window, are associated with a high diagnostic accuracy, low morbidity and mortality, and a high rate of success.[21] These procedures are the treatment of choice for persistent symptomatic effusion that is unresponsive to medical management and for tamponade from constrictive pericarditis. Pericardial fluid should be sent for protein assessment, cell counts, Gram's stain, culture, and cytology. Instillation of sclerosing agents should be reserved for palliation in patients with recurrent or refractory tumor. In patients with a Wilms' tumor thrombus extending into the right side of the heart, if the mass does not occlude the chambers, therapy with dactinomycin and vincristine may reduce the size of the thrombus within a week (see Chapter 30).

## Massive Hemoptysis

### *Etiology and Evaluation*

Massive blood loss into the respiratory tree can cause thrombus formation within the bronchial tree, leading to asphyxiation, or less likely, exsanguination. In general, pediatric tumors do not cause massive hemoptysis. The most common etiology of mild hemoptysis in an oncology patient is aspiration of blood from epistaxis, whereas the most common cause of massive hemoptysis is invasive pulmonary aspergillosis. The incidence of hemoptysis in pulmonary aspergillosis ranges from 2% to 26%.[23] The chest radiograph may show a nodular or cavitary lesion, a peripheral wedge-shaped infiltrate, or disseminated infiltrates. A CT scan should be obtained to determine the extent of fungal disease (Fig. 39-3). The differential diagnosis includes all invasive fungi and, less frequently, bacterial pneumonias and consolidations with such invasive organisms as *Staphylococcus aureus* and *Klebsiella* and *Pseudomonas* species. A CBC, along with prothrombin time, partial thromboplastin time, and assessment of fibrinogen and fibrin split products should be obtained immediately to look for coagulation abnormalities.

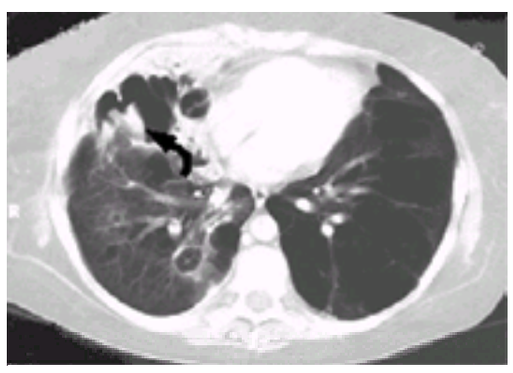

**FIGURE 39-3.** Computed tomographic image of the chest viewed at lung windows shows a fungus ball (*curved arrow*) within an air cyst in a 20-year-old woman with Langerhans' cell histiocytosis. Note extensive cystic disease and parenchymal destruction in both lungs. (Courtesy of James S. Meyer, M.D., Children's Hospital of Philadelphia.)

### *Therapy*

Therapeutic objectives are to prevent asphyxiation, localize the site of the bleeding, and arrest hemorrhage. If the site of bleeding is known, the patient should lie on the side of the hemorrhage to prevent collection of blood into the normal lung. Preventing asphyxiation may require intubation. Oropharyngeal tubes should be large enough to allow passage of a bronchoscope.[22] Nasotracheal intubation may cause epistaxis, making localization of the bleed difficult.[22] Thrombocytopenia or a coagulopathy should be corrected and erythrocytes transfused as needed. When the patient has been stabilized, a chest radiograph should be obtained and bronchoscopy undertaken if the hemoptysis persists. If the source of bleeding is identified transcatheter embolization (selective intrabronchial coagulation with a hemostatic complex consisting of the fibrin precursors, calcium chloride, and thrombin) or occlusion of the hemorrhaging vessel with a balloon catheter have proven successful in patients with massive pulmonary hemorrhage from causes other than *Aspergillus* infection.[23,24,25 and 26] Unfortunately, these measures are unlikely to be successful in massive hemoptysis from *Aspergillus*, with its extensive angioinvasion and necrosis. Shapiro et al.,[27] using a subcutaneous No. 12 French Ring-McLean sump catheter, first to aspirate the cavity and then to instill *N*-acetylcysteine (10% solution, 2 mL in 20 mL of sterile saline) and 40 mg amphotericin B, report successful treatment of massive hemoptysis in six patients with localized aspergillomas.[27] Instillations were separated by 8 hours.

In most cases of hemoptysis, the bleeding eventually stops. In a patient with known fungal disease experiencing recurrent episodes of hemoptysis, the lesion should be widely excised, which may involve resection of a bronchopulmonary segment or a lobectomy. Medical management of mild hemoptysis from pulmonary aspergillosis is possible as cavities seal off and form linear scars (see Chapter 41). If medical management is undertaken, it is prudent to manage the patient in the hospital until the hemoptysis has resolved and the lesion is regressing. If an undiagnosed malignancy is found to be the cause of the hemoptysis, surgical resection is indicated when possible. Initiating chemotherapy or emergent radiotherapy can also improve symptoms rapidly.

### Pneumothorax or Pneumomediastinum

#### *Etiology and Evaluation*

A *pneumothorax* is air in the pleural space; *pneumomediastinum* is air in the mediastinum, often with subcutaneous emphysema. A tension pneumothorax arises when inspired air accumulates in the pleural space and is not expelled with expiration due to a one-way valve effect. A tension pneumothorax eventually will cause mediastinal shift and circulatory collapse.[28]

A pneumothorax or pneumomediastinum is a rare presenting symptom of an undiagnosed malignancy. Either is more likely due to infection, chemotherapy-induced emesis, esophageal perforation, recurrent or metastatic disease, pulmonary fibrosis from radiation or bleomycin, pulmonary histiocytosis, or idiopathic causes.[29,30] and [31]

Patients present with cough, dyspnea, or pleuritic chest pain, but often a pneumothorax is found incidentally on chest radiography. Examination may show decreased breath sounds on the affected side, tachycardia, tachypnea, shifting of the trachea, hyperresonance to percussion, or subcutaneous emphysema.[28] A chest radiography may show a collapsed lung. In pneumomediastinum, air can be seen tracking through the soft tissue and under the skin.[30] Shifting of the cardiac shadow and trachea indicates a tension pneumothorax that must be relieved immediately.[28] Pulse oximetry or an arterial blood gas evaluation can be used to assess oxygenation. CT with contrast esophagography can rule out esophageal perforation as a cause of pneumomediastinum.[30]

#### *Treatment*

The patient should immediately be placed on 100% oxygen. If the lesion is small, oxygen may suffice. If the lesion is large or a tension pneumothorax or pneumomediastinum is present, the excess pleural or mediastinal air must be evacuated.[28] Needle thoracentesis can be performed immediately, but a chest tube should be placed for long-term evacuation.[29] Tension pneumomediastinum can be relieved using a subxiphoid incision and placement of a chest tube retrosternally.[30] In the case of recurrent pneumothoraces, pleurodesis with mechanical abrasion or a chemical agent (see the section Pleural and Pericardial Effusions) can prevent recurrence. Treatment of the underlying problem, such as antibiotics for infections or steroids for pulmonary fibrosis, should be initiated as soon as the patient is stabilized.[30]

### Retinoic Acid Syndrome

#### *Etiology and Pathophysiology*

All-*trans*-retinoic acid (ATRA) is used to treat acute promyelocytic leukemia (APML), but up to 26% of patients develop the retinoic acid syndrome 2 days to several weeks after starting ATRA.[32] Overall mortality ranges from 5% to 13%.[32] Manifestations include unexplained fever, respiratory distress, weight gain, fluid retention, pleural and pericardial effusions, hypotension, and renal failure. The syndrome only occurs during induction therapy and appears to be unique to patients with APML.[32]

The onset of symptoms often coincides with the development of hyperleukocytosis that occurs as a result of the ATRA-induced proliferation and differentiation of leukemic promyelocytes. Autopsy shows pulmonary interstitial infiltration with maturing myeloid cells and endothelial cell damage. The pathogenesis is unknown. As the retinoic acid syndrome has been described in patients with low peripheral WBC counts, it is unlikely to be only a result of the mechanical effects of high numbers of leukocytes. Release of cytokines that enhance capillary permeability, promote leukocyte aggregation, and up-regulate leukemic cell binding to the endothelium may contribute to the retinoic acid syndrome.

#### *Evaluation and Differential Diagnosis*

The retinoic acid syndrome should be considered in any patient who develops respiratory distress while receiving ATRA. Chest radiography may reveal pulmonary edema or infiltrates and a pleural or pericardial effusion. The differential diagnosis includes pneumonia or sepsis with bacteria or fungi, congestive heart failure, acute respiratory distress syndrome, or any other cause of interstitial pneumonitis.

#### *Therapy*

If the retinoic acid syndrome develops, dexamethasone, 10 mg intravenously every 12 hours in adults and 0.5 to 1.0 mg per kg per dose every 12 hours in children, often reverses the process.[32] ATRA and other chemotherapeutic agents may be withheld if the symptoms are life threatening. When the symptoms resolve, ATRA therapy may resume at a reduced dose and under close observation. The dose can be slowly escalated back to full dose over several days.[32]

## ABDOMINAL EMERGENCIES

In the 1970s and early 1980s, abdominal emergencies in cancer patients were managed with intensive medical therapy and avoidance of surgical intervention; the outcome, however, was poor.[33,34] More recently, judicious use of surgery has led to better overall results.[34] Today advances in diagnostic imaging, interventional radiology, endoscopy, and laparoscopy allow earlier diagnosis with less invasive management overall. Even when an acute abdomen in a child with cancer appears to be treatable with medical management alone, an early surgical consultation is advisable.

### Etiology and Pathophysiology

Although a child with cancer can develop an acute abdomen for the same reasons as can any other child, an acute abdomen unrelated to the underlying malignancy or its treatment is unusual.[34] Many uncommon abdominal emergencies occur commonly in the immunocompromised host, including esophagitis, gastric hemorrhage, typhlitis, perirectal abscess, hemorrhagic pancreatitis, and massive acute hepatic enlargement from tumor.[35,36]

Abdominal emergencies in the child with cancer arise because of hemorrhage, mechanical obstruction, perforation, and inflammation.[34,37,38] Hemorrhage may result from thrombocytopenia, coagulopathy, mucosal ulceration, or abnormal tumor vessels. Obstruction results from compression of a gastrointestinal lumen by a tumor or abscess or from therapy-induced ileus. Perforation can result from unresolved obstruction, localized ulceration, and segmental necrosis. Abdominal processes that are localized in healthy children may be generalized in the neutropenic or immunosuppressed child. Moreover, the inflammatory response may be blunted by a lack of WBCs. Wound healing may be slow because of the effects of malnutrition and chemotherapeutic agents, notably corticosteroids, on fibroblast proliferation and collagen production.[39]

### Evaluation and Differential Diagnosis

Pain is the principal symptom of an acute abdominal process, regardless of the patient's state of compromise ( Table 39-3).[36] Determination of the location, quality, and timing of pain in relation to status of the cancer, recent medications, and surgical history is important. Gastrointestinal hemorrhage is a sign of a serious intra-abdominal process. Changes in vital signs (including fever, hypertension or hypotension, and tachycardia), the presence of blood in vomitus or stool, abdominal distention, or absence of flatus or stool are less specific indications of an acute abdomen. At the same time, the practitioner must remember that in an immunosuppressed patient, fever does not always accompany infection.

| |
|---|
| Constipation/obstipation |
| Vincristine |
| Narcotics |
| Amitriptyline |
| Distention |
| Ascites |
| Primary tumor |
| Lymphoma |
| Sarcoma |
| Neuroblastoma |
| Wilms' tumor |
| Germ cell tumors |
| Hepatosplenomegaly |
| Nerve compression |
| Retroperitoneal nodes |
| Inflammation |
| Colitis/typhlitis |
| Ulceration |
| Referred pain |
| Pneumonitis |
| Pleural or pulmonary tumor |
| Retroperitoneal tumor |

**TABLE 39-3. DIFFERENTIAL DIAGNOSIS OF ABDOMINAL PAIN IN PEDIATRIC CANCER PATIENTS**

The practitioner should perform the examination in a manner that causes the least discomfort possible. Encouraging the child to participate may make the examination easier. On observation, one should note whether the child lies still, is willing to move, or winces with cough, movement, or motion of the bed. Observation may also reveal distention, asymmetry, and surgical scars. Auscultation can help to differentiate ileus from obstruction. Complete absence of bowel sounds or waves, rushes, and paroxysms are more likely indications of obstruction. An occasional tinkle or drip-drop plunking sound is often heard when an ileus is present. Compressing the head of the stethoscope gently into the abdomen during auscultation can reveal guarding or tenderness.

Inspection of the perineum and anus is important in the febrile neutropenic patient. Although rectal examination is mandatory to evaluate obstruction and peritonitis even in a neutropenic patient, such an examination should be performed once by an experienced examiner. Thrombocytopenia, neutropenia, and pain are not contraindications to a gentle rectal examination (e.g., using the fifth finger in infants). Pain detected on rectal examination may be the result of generalized serositis, mucositis, or abscess.

## Laboratory and Diagnostic Studies

Selective use of laboratory and radiographic studies may distinguish the child who requires immediate surgical intervention from one who will respond to medical management. Serial CBCs can help monitor hemorrhage and reveal neutropenia that interferes with pus and abscess formation. Blood cultures that are persistently positive despite a patient's treatment with appropriate antibiotics may indicate the presence of an abscess or necrotic bowel. Electrolyte changes may reveal metabolic deficits from fluid shifts before such deficits are clinically apparent. Traditionally, abdominal paracentesis and diagnostic lavage were performed to give information about extraluminal bleeding, intestinal perforation, or infection. These techniques are being replaced by laparoscopy, which permits therapeutic intervention in addition to offering diagnostic potential. [40]

Diagnostic radiologic studies begin with supine, erect, and left lateral decubitus abdominal films. [41,42] Obstruction, especially high small-bowel obstruction, pneumatosis intestinalis, and intra-abdominal free air, can be diagnosed by these studies. A chest radiograph may reveal pneumonia as the etiology of upper quadrant abdominal pain. Barium studies may help to diagnose esophagitis or colitis, but direct examination with endoscopy or colonoscopy has replaced contrast studies in the evaluation of the gastrointestinal tract in both immunocompromised and healthy children. [42] Abdominal ultrasonography (US), CT, magnetic resonance imaging (MRI), and angiography all may be used to locate and characterize mass lesions. In a large retrospective study, the presence of peritoneal signs on examination and pneumatosis intestinalis on radiography were highly associated with the presence of an acute surgical process in children receiving chemotherapy. [34]

## Hemorrhage

### *Esophageal Varices*

Esophageal varices can develop as a consequence of fibrosis, cholangitis, and cirrhosis. They occur in patients with Langerhans cell histiocytosis, refractory abdominal tumors compressing the portal vein, or chronic viral hepatitis. Variceal bleeding is brisk, and patients may have associated hematemesis and melena. A CBC, prothrombin time, partial thromboplastin time, and blood type and cross-match should be obtained immediately. Initial management includes elevation of the head of the bed to 30 to 45 degrees, judicious volume expansion with normal saline or Ringer's lactate solution, and correction of anemia. Overexpansion may contribute to continued bleeding, rebleeding, thrombocytopenia, and coagulation abnormalities. [43] Febrile patients should receive broad-spectrum antibiotics, as bleeding varices may be a sign of sepsis in a patient with cirrhosis. Passage of a nasogastric tube and gastric lavage may be initiated as follows: 50-mL volume of normal saline at room temperature through a 12 French tube in infants, and 100 to 200 mL through a 14 French tube in older children. [43] Lavage should continue for 5 to 10 minutes, and a record should be kept of volumes in and out. [43]

Emergent management is indicated if bright red blood persists in the lavage fluid. Systemic infusion of vasopressin through a large-bore intravenous line or CVL decreases blood flow through the portal circulation. Vasopressin infusion rates are as follows: in children younger than 5 years, 0.1 unit per minute, increasing hourly by 0.05 units per minute to a maximum of 0.20 units per minute; in children aged 5 to 12 years, 0.30 units per minute; and in those children older than 12 years, 0.40 units per minute.[43] Infusion should continue for 12 to 24 hours, with cardiac monitoring for evidence of myocardial ischemia or arrhythmias and frequent observation for signs and symptoms of limb ischemia. If bleeding persists, endoscopy with endoscopic variceal ligation or sclerotherapy may be useful, preferably performed within hours of the onset of bleeding. [43] Balloon tamponade under direct observation with a Sengstaken-Blakemore tube or Linton tube may control refractory bleeding but is associated with considerable morbidity and mortality. [43] Splenorenal shunts are a last resort.

### *Upper Gastrointestinal Hemorrhage*

Hematemesis and melena may consist of blood swallowed from epistaxis or oropharyngeal mucositis or may signify upper gastrointestinal hemorrhage. Children with cancer are prone to multiple punctate, shallow gastric and duodenal ulcers (i.e., stress ulcers, Cushing's ulcers). Children taking high-dose corticosteroids, those with increased intracranial pressure, and those who underwent high-dose irradiation are at especially high risk of gastritis and ulcers. [44,45] Chemotherapy-induced emesis can cause Mallory-Weiss tears, which are mucosal lacerations at the gastroesophageal junction resulting from large gradients between the intragastric and intrathoracic pressure incurred during emesis.

Children with increased intracranial pressure receiving steroids for posterior fossa tumors and any patient on long-term steroid therapy should be treated empirically with antacids or an $H_2$-blocker (ranitidine, 1.0 to 1.5 mg per kg intravenously every 6 hours or 2 mg per kg orally twice daily). [43] Medical management of bleeding consists of $H_2$-blockers, antacids, lavage, and correction of thrombocytopenia and coagulation abnormalities, as described for esophageal varices. Endoscopy is indicated for uncontrolled or recurrent bleeding, and hemostasis may be achieved with a heater probe or multipolar electrocoagulation. [43] Sclerotherapy is not indicated. Surgery for persistent bleeding must be individualized but most often entails vagotomy, pyloroplasty, and oversewing of the ulcer.

### *Lower Gastrointestinal Hemorrhage*

Typhlitis resulting from infection with *Clostridium difficile,* cryptosporidium, and fungi are associated with bloody stools. Intussusception due to tumor, surgery, or an abscess can cause intermittent bleeding or “currant-jelly” stools. Most major lower gastrointestinal hemorrhage in the pediatric cancer patient is multifactorial (e.g., *C. difficile* enterocolitis in a child with thrombocytopenia and coagulopathy). [45] Hemorrhoids and anal fissures occasionally cause bleeding in children with cancer. Rarely is the bleeding significant, often being reported as blood in the toilet bowl or on the toilet paper. Management is directed at each of the underlying causes.

## Gastrointestinal Obstruction

The differential diagnosis of bowel obstruction in the child who has had adjuvant therapy or previous abdominal surgery includes obstipation or paralytic ileus induced by vinca alkaloids, narcotics, or tricyclic antidepressants, adhesions or strictures, and intussusception. Small-bowel intussusception usually occurs days to weeks

postoperatively (Fig. 39-4).[46] Although rare in children, bowel obstruction from primary cancer may be the presenting symptom of a Burkitt's lymphoma of the ileum. It may also complicate palliative care in patients with refractory sarcomas, particularly desmoplastic small-cell sarcoma, or with typical adult colon or ovarian stromal cell cancer. Presacral teratomas and pelvic sarcomas may occlude the rectum.

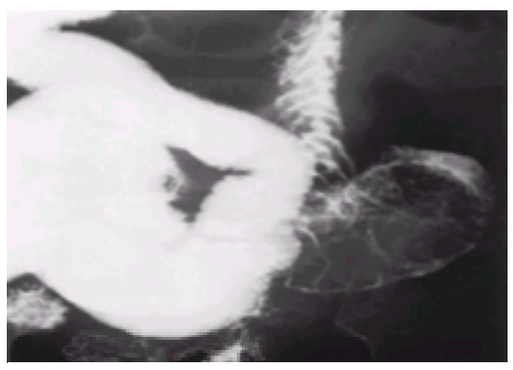

**FIGURE 39-4.** Contrast study of a 3-year-old girl 6 days after removal of a Wilms' tumor shows evidence of complete small-bowel obstruction. The study illustrates a filling defect from small-bowel intussusception.

A patient history, physical examination, and plain-film radiographs that document obstipation can help to differentiate between narcotic or vincristine-related ileus and a surgical abdomen. Small-bowel obstruction is managed initially by taking a patient off anything administered by mouth and placing a nasogastric tube for decompression. A Miller-Abbott or other long intestinal tube alone may be indicated immediately postoperatively, but its use is unwarranted in the child who has had an abdominal procedure in the distant past. Evaluation for the underlying etiology should proceed as described in the section Laboratory and Diagnostic Studies. Use of a stool softener and passage of a small, well-lubricated rectal tube may relieve rectal obstruction until cytotoxic therapy reduces the tumor mass.

### Gastrointestinal Perforation

Perforation may be the outcome of unresolved obstruction, ulcers, or gastritis unresponsive to medical therapy, infections such as typhlitis, or erosion by the primary tumor.[36] In the case of abdominal Burkitt's lymphoma, perforation may occur at presentation, during steroid therapy, or after clinical resolution in association with lysis of a penetrating metastasis. Abdominal radiographs may reveal air under the diaphragm, tracking into the liver and along the flank. Perforations are a surgical emergency and are managed either by resection of the affected area and secondary closure or by primary reanastomosis.[36] In massively disseminated abdominal Burkitt's tumor, cytotoxic therapy may be necessary to reduce the tumor burden before surgical access is possible.

### Gastrointestinal Infection and Inflammation

Fungal or viral esophagitis, the most common esophageal problem in children with cancer, is discussed in Chapter 41. Neutropenic enterocolitis, typhlitis, and appendicitis have very similar presentations in children with cancer. Occasionally, the more commonplace abdominal emergencies in healthy children, such as appendicitis, are overlooked in the oncology patient. At St. Jude Children's Research Hospital, 16 cases of appendicitis were seen among 6,099 children with cancer (0.5%).[47] Fourteen of the cases occurred among the 2,794 children with acute leukemia.[47] The clinical diagnosis was delayed in 37.5%, with typhlitis being the most common confounding consideration. In diagnosing appendicitis, both US and CT scans are superior to clinical evaluation alone.[42,48]

Whereas appendicitis may be the cause of right lower quadrant pain in the child with cancer, in the child with prolonged neutropenia, typhlitis is the major concern. Typhlitis is a necrotizing colitis localized to the cecum. Fever usually is present, although this is an undependable sign. Typhlitis usually occurs subsequent to cytotoxic chemotherapy, but one 3-year-old child had typhlitis on presentation of ALL.[49] Shamberger et al.[50] found that one-third of patients with acute myelogenous leukemia (AML) had documented typhlitis during induction therapy.

Bacterial or fungal invasion of cecal mucosa may progress from inflammation to full-thickness infarction and perforation. *Pseudomonas* species, *Escherichia coli*, and other gram-negative bacteria, *S.aureus*, alpha-hemolytic streptococci, and *Clostridium* species are the common bacterial pathogens. *Candida* and *Aspergillus* species are the major fungal pathogens.[49,50,51,52] and [53] Fungus was found at postmortem examination in 24% of patients with leukemia and typhlitis.[53]

Radiographic studies may demonstrate pneumatosis intestinalis or nonspecific thickening of the bowel wall. In a retrospective review of 24 children with typhlitis, Sloas et al.[52] found that CT and US were more sensitive than plain-film radiography: False-negative rates were 15% for CT, 23% for US, and 48% for plain-film radiography. Figure 39-5 exhibits the appearance of typhlitis on a CT scan.

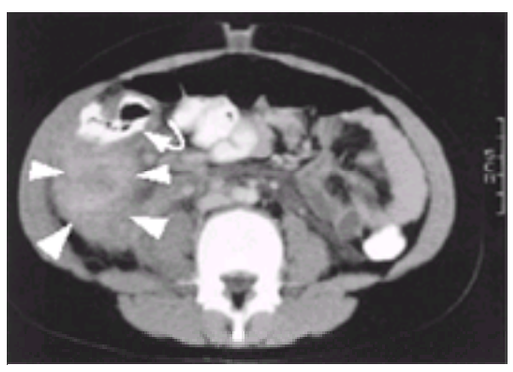

**FIGURE 39-5.** Computed tomographic scan showing a poorly defined soft tissue mass and indicating a phlegmon (*arrowheads*) and thickened cecal wall (*curved arrow*) in a patient with typhlitis. (Courtesy of Dr. James Meyer, Children's Hospital of Philadelphia.)

In the past, the mortality rate from typhlitis ranged from 20% to almost 100% with either surgical or medical treatment.[49,50,52,54] Shamberger et al.[50] have proposed four criteria for surgical intervention in typhlitis: (a) persistent gastrointestinal bleeding despite resolution of thrombocytopenia and correction of clotting abnormalities; (b) evidence of free air; (c ) need for vasopressor support or large volumes of fluid, suggesting uncontrolled sepsis from intestinal infarction; and (d) development of symptoms of an intraabdominal process that would normally require an operation. Pneumatosis and localized peritoneal signs are not sufficient to warrant surgical exploration in the absence of one or more of the preceding indications. Using these criteria, 70% to 80% of patients can be managed medically with broad-spectrum antibiotics to cover gram-negative pathogens and clindamycin or metronidazole for gastrointestinal anaerobes and fungi.[52]

Patients who do not have typhlitis but who demonstrate signs and symptoms of enterocolitis may have transverse colitis, intussusception, or antibiotic-related pseudomembranous or clostridial enterocolitis.[55] *C. difficile* colitis is now the major cause of nosocomial diarrhea in the United States and Western Europe.[56] Although use of second- and third-generation cephalosporins, clindamycin, ampicillin, and amoxicillin are associated with the highest risk of *C. difficile* colitis, even a single dose of almost any antibiotic may predispose the patient to this condition.[56] The specific therapy for *C. difficile* colitis is oral metronidazole (5 mg per kg every 6 hours, up to a maximum of 4 g per day) or oral vancomycin (125 mg four times daily or 50 mg four times daily in patients weighing less than 30 kg).[56]

### Perirectal Abscess

Anorectal pain, tenderness, and discomfort with bowel movements may indicate a perirectal abscess or fistula. Perirectal abscesses occur in patients with neutropenia, especially those with AML.[57] Superficial lesions are obvious; deeper lesions require cautious rectal examination. In a neutropenic patient, the only physical finding may be tender, brawny, woody edema with a dense cellulitic reaction. Most abscesses are caused by a mixture of aerobes, including staphylococci, *E. coli*, pseudomonads, and streptococci, enterococci, and other fecal anaerobes.[58] Initial therapy includes antibiotics to cover aerobic and anaerobic gram-negative and gram-positive bacteria. Sitz baths may relieve pain. If the abscess or induration is well-circumscribed or progresses, incision and drainage of the lesion may be necessary.[57,59] Early, aggressive medical management often eliminates the need for incision and drainage, particularly while the patient is neutropenic. Follow-up evaluation of the patient is important, but repeated rectal examinations should be avoided.

### Cholecystitis and Biliary Obstruction

Inflammation of the liver and biliary tract can present as localized right upper quadrant pain or jaundice. Acute cholecystitis and particularly acute acalculous cholecystitis occur in children who are septic, stressed, and volume-depleted. Biliary obstruction as a result of primary tumor is rare: Lymphoma and neuroblastoma occasionally block biliary flow, and rhabdomyosarcoma of the common duct occurs.[60]

US or CT can distinguish calculous from acalculous cholecystitis.[61] Hydration, broad-spectrum antibiotics, and nasogastric decompression usually treat the acute cholecystitis. Antibiotics should cover gram-negative bacilli and, in the neutropenic patient, fungi. Endoscopic or combined endoscopic-cutaneous decompression is successful in most patients, and stent placement may provide effective palliation for up to 6 months.[62,63]

### Veno-Occlusive Disease

Veno-occlusive disease consists of rapid, usually painful hepatic enlargement, more than a 5% weight gain, ascites, and hyperbilirubinemia that occurs most frequently within the first 2 weeks of cytoreductive therapy for bone marrow transplantation. Weight gain in excess of 15% and serum bilirubin in excess of 10 mg per dL by day 10 are harbingers of multisystem organ failure.[64] Recently, cases of veno-occlusive disease have occurred during 6-thioguanine therapy for ALL and after vincristine and dactinomycin with or without cyclophosphamide therapy for rhabdomyosarcoma and nephroblastoma.[65,66] and [67] Treatment of veno-occlusive disease is primarily supportive. In the nontransplant setting, 6-thioguanine, vincristine, or actinomycin should be withheld until symptoms improve.

### Acute Massive Hepatomegaly in Neuroblastoma

Massive hepatomegaly may complicate stage IV-S neuroblastoma in the neonate and may be fatal.[68] Unless life-threatening respiratory embarrassment is present, supportive care and observation are sufficient until the disease resolves spontaneously. If hepatomegaly compromises respiratory function, the therapeutic options include chemotherapy, irradiation and, rarely, surgical enlargement of the abdominal wall with a Silastic pouch.[69] Radiotherapy involves lateral portals (with the child positioned supine) and fields encompassing most of the liver but sparing the ovaries and kidneys and extending from the dome to the posterior portion of the right hepatic lobe. A midplane dose of 150 cGy given on three successive days is the standard at the Children's Hospital of Philadelphia.

### Hemorrhagic Pancreatitis

When vomiting and abdominal pain occurs in a child receiving L-asparaginase or corticosteroids or in a child with increased intracranial pressure, pancreatitis should be included in the differential diagnosis.[70,71] and [72] Standard-risk ALL therapy is associated with a 2% incidence of pancreatitis.[73] Serum chemistries, including pancreatic amylase and lipase and, in some situations, the urinary amylase-creatinine ratio, help to establish the diagnosis. A US or CT scan of the pancreas should be obtained. Randomized trials of nasogastric tubes for suction or to allow resting of the pancreas have not proven such instrumentation to be beneficial unless the patient has intractable emesis or an ileus.[74] Fluid loss should be replaced, and antibiotics appropriate for the immunologic status of the patient should be started. Imipenem may be of specific benefit in necrotizing pancreatitis.[74] Parenteral nutrition may be necessary.[75] Recent reports suggest that octreotide (5 µg per kg per day divided into twice-daily doses) may ameliorate the clinical course of hemorrhagic pancreatitis.[76,77]

The indications for and timing of surgical drainage of a pancreatic abscess depend on the child's condition and the evolution of the process on sequential US or CT examinations.[42,78] Although an area of phlegmon can be treated conservatively, localized abscess and pancreatic dissolution require emergent open drainage. A CT scan with contrast medium may show a phlegmon as a diffuse homogeneous area, and an abscess may appear as a loculation or loculations of various densities within the pancreas.[42] If pancreatitis evolves into a pseudocyst, supportive care permitting maturation of the cyst beyond 6 weeks often facilitates effective internal drainage.[71,79] Percutaneous CT-guided drainage may also be effective.

## GENITOURINARY EMERGENCIES

Renal complications in children with malignancies can be divided into primary complications caused by the tumor itself, such as obstruction of the urinary tract and renal vein thrombosis, and complications that are secondary to the therapy, such as hemorrhagic cystitis.[80] Acute renal failure, hypertension, and TLS can be both primary and secondary. A discussion of TLS is included in the section Metabolic Emergencies.

### Oliguria and Anuria

In a child whose urine output is reduced or absent, it is necessary to determine whether the cause is prerenal, renal, or postrenal. Septic shock is the most common prerenal cause of reduced urine output in children receiving cancer treatment. Chemotherapy-induced emesis, profuse infectious diarrhea, negligible oral intake, and metabolic abnormalities all can cause intravascular depletion and oliguria.[80] Bulky abdominal or pelvic tumors, including retroperitoneal sarcomas and lymphomas, germ cell tumors, stromal ovarian tumors, and adrenal or celiac axis neuroblastomas may compress or obstruct the ureters or bladder, causing postrenal failure (Fig. 39-6).[80] Chemotherapy, antibiotics, and antifungals can cause renal insufficiency.[80] Narcotics, vincristine, phenothiazines, and herpes zoster can affect the sacral nerves and cause temporary urinary retention.

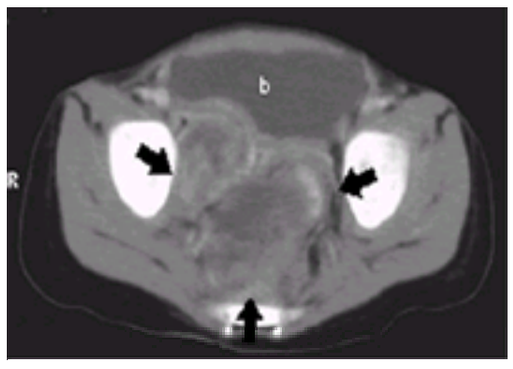


**FIGURE 39-6.** Computed tomographic image of the pelvis viewed at soft tissue windows shows lobulated presacral mass (*arrows*), displacing the bladder (b) anteriorly in a 2-year-old girl with a sacrococcygeal teratoma. Masses in these locations often compress the ureters and cause upper urinary tract obstruction. (Courtesy of James S. Meyer, M.D., Children's Hospital of Philadelphia.)

In a child presenting with a new abdominal or pelvic mass, the practitioner should inquire about urinary or bowel dysfunction and should obtain blood urea nitrogen (BUN) and creatinine (Cr) levels. CT or US of the abdomen and pelvis can reveal both a large mass and renomegaly.[81] If there is no evidence of impending renal failure, contrast may be used. Delayed filling of the bladder may be noted. Postrenal oliguria from mass effects must be differentiated from TLS, as both can present

with elevated uric acid, potassium, and phosphorus, although very elevated BUN and Cr levels are more consistent with postrenal failure.

Vigorous hydration quickly corrects oliguria in patients with prerenal failure but should be avoided in postrenal failure. In most cases of obstructive uropathy, the placement of a urinary catheter past the obstruction or surgical placement of ureteral stents will lead to rapid improvement. Treatment of the underlying tumor with surgery, irradiation, or chemotherapy will ultimately relieve the obstruction. Nephrotoxic drugs may have to be stopped and replaced with less toxic agents.

### Hypertension

#### *Etiology and Differential Diagnosis*

Hypertension is defined as systolic or diastolic blood pressures exceeding the ninety-fifth percentile for age and gender. [82] Symptoms include headache, irritability, lethargy, confusion and, ultimately, seizures and coma. Hypertension can be due to renal artery compression or renal parenchymal compression leading to increased renin production.[83] These most commonly occur in Wilms' tumor, neuroblastoma, ganglioneuroblastoma, abdominal lymphomas, and pheochromocytomas. Of note, Wilms' tumors and neuroblastomas can produce renin ectopically. [83] Renal vein thrombosis can cause arterial systolic hypertension and hematuria. [84] Cushing's triad, consisting of hypertension, bradycardia, and respiratory depression, indicates increased intracranial pressure in a child with a brain tumor, central nervous system (CNS) leukemia, or a CNS infection. Hypertension can also be a secondary effect of medications including steroids, cyclosporin A, and amphotericin B. Finally, pain alone can cause significant hypertension in children, often associated with tachycardia.

#### *Evaluation*

Vital signs including frequent blood pressure monitoring should be obtained. Crying, fever, or pain may temporarily elevate the blood pressure. Signs of increased intracranial pressure warrant a CT scan of the brain. Urine and plasma catecholamine levels and plasma renin levels may be elevated in paraneoplastic hypertension.[83] Radiologic evaluation should include CT of the abdomen to rule out an underlying malignancy and Doppler US to evaluate renal blood flow.

#### *Therapy*

Treatment of hypertension typically results in rapid improvement of neurologic sequelae. A modest reduction in blood pressure can prevent stroke, encephalopathy, and congestive heart failure. Excessively rapid reduction in blood pressure reduces perfusion of end organs. Recommended intravenous therapy for patients with normal renal function includes nitroprusside (0.5 to 8.0 µg per kg per minute by infusion), diazoxide (2 to 5 mg per kg per dose intravenously), and hydralazine (0.2 to 0.4 mg per kg per dose intravenously).[82] If a patient is fluid overloaded, furosemide (0.5 to 1.0 mg per kg) is indicated. Sublingual nifedipine (5 to 10 mg per dose for children weighing more than 10 kg) acts rapidly and is especially useful to treat asymptomatic hypertension. A long-acting angiotensin-converting enzyme inhibitor or calcium channel blocker dosed once or twice daily provides long-term control. [82] For hypertension caused by increased intracranial pressure, dexamethasone or mannitol can decrease cerebral edema, in turn decreasing the blood pressure (see the section Neurologic Emergencies). Definitive treatment of hypertension caused by the tumor consists of treatment of the tumor with cytotoxic therapy.

### Hemorrhagic Cystitis

#### *Etiology and Evaluation*

Signs and symptoms of hemorrhagic cystitis are dysuria, urgency, and frequency, with leukocytes, erythrocytes, or clots found in the urine because of bleeding and inflammation of the bladder. Hemorrhagic cystitis can cause substantial blood loss and urinary obstruction. In the immunosuppressed patient, adenovirus, cytomegalovirus, and polyomavirus BK can incite hemorrhagic cystitis.[85] Therapy with cyclophosphamide or ifosfamide is the most common cause of this disease. [85] Acrolein, the principal metabolite of these two drugs, is toxic when it precipitates in the bladder. [85] The early phases of cystitis are mucosal edema, ulceration, epithelial necrosis, and submucosal fibrosis.[85,86] Cystitis may occur hours to months after cyclophosphamide or ifosfamide administration. Long-term complications include bladder fibrosis and contraction, urinary reflux, renal failure, and transitional cell bladder tumors. [85,86]

The diagnosis is made by history and urinalysis. Patients usually present with gross hematuria and the passing of painful clots. US may demonstrate a boggy, edematous, hemorrhagic bladder or, possibly, a fibrotic bladder with hemorrhage. Direct examination may be necessary to locate large areas of bleeding.

#### *Therapy*

Chemotherapy-induced cystitis is best prevented by vigorous hydration and brisk diuresis to reduce accumulation of acrolein in the bladder during cyclophosphamide or ifosfamide infusion.[86] Concurrent use of the uroprotective agent sodium 2-mercaptoethane sulfonate (mesna), which binds acrolein, has decreased the need for prophylactic bladder irrigation and hyperhydration. [86]

If prevention fails, immediate treatment consists of hydration, correction of thrombocytopenia and coagulation abnormalities, transfusion of packed red blood cells, and placement of a double-lumen Foley catheter for continuous bladder irrigation. Bladder spasms may be controlled with oral oxybutynin chloride (5 mg twice daily in children older than 5 years), baclofen (3 to 5 mg every 8 hours orally, titrated up every 3 days), belladonna, or opioids. Concurrent bladder irradiation and chemotherapy with radiomimetic agents should be stopped. Patients who continue to bleed may need endoscopy and electrocoagulation. If electrocoagulation fails, instillation of formalin, alum, or prostaglandin $E_2$ ($PGE_2$) may be helpful. Traditionally, the treatment was a 0.25% solution of formalin instilled into the bladder through a Foley catheter while the patient was anesthetized.[85,87] The risks of formal in include obstruction, extravasation, and bladder constriction. In patients with reflux, formalin is contraindicated and alum instillation is preferred. A newer, less toxic therapy is $PGE_2$. The instillation of $PGE_2$ resulted in resolution of hematuria in all patients within 5 days of infusion without any systemic side effects.[88]

## NEUROLOGIC EMERGENCIES

Neurologic emergencies in children with cancer can be a direct effect of the malignancy, as with spinal cord compression in neuroblastoma or increased intracranial pressure from a CNS tumor, or they can result indirectly from abnormalities of metabolism, hemostasis, or organ system dysfunction. Altered mental status, seizures, cerebellar dysfunction, and cerebrovascular accidents (CVAs) may be the sequelae of cancer therapy or supportive care agents.

A common mistake in evaluating a child with cancer and acute neurologic deterioration is the omission of a careful, detailed neurologic examination. The child often is considered too weak, too uncooperative, or in too much pain to be put through a formal neurologic evaluation. The reason for the child's misery frequently lies in the CNS impairment itself. Without neurologic localization of the deficit, unnecessary, potentially harmful investigations may be carried out, further delaying diagnosis and possibly increasing morbidity and mortality.

### Acute Alterations in Consciousness

#### *Etiology and Pathogenesis*

Table 39-4 lists the causes of acute alterations in consciousness (AAC) in the child with cancer. Given the myriad etiologies of AAC in the ill, unstable child with cancer, a comprehensive evaluation is necessary to determine a specific cause.

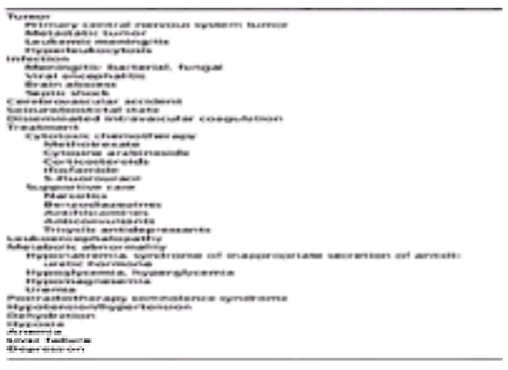


**TABLE 39-4. ETIOLOGY OF ACUTE ALTERATIONS IN CONSCIOUSNESS IN CHILDREN WITH CANCER**

A state of full awareness depends on the integrity of the cerebral hemispheres and the ascending reticular activating system (ARAS), a polysynaptic neuronal network lying in the central core of the brainstem, thalamus, and hypothalamus.[89] The ARAS receives input from and projects to all portions of the CNS. AAC range from lethargy, in which the patient has difficulty maintaining an aroused state, to coma, in which the patient is unresponsive to all pain and stimuli. Coma or other forms of altered consciousness result from diffuse derangement of cerebral function or damage to the ARAS. Drugs usually cause alterations in consciousness by derangements of diffuse bilateral cerebral function. Increased intracranial pressure from a localized mass may cause herniation or compression of the brainstem and disruption of the ARAS.[89]

***Evaluation and Differential Diagnosis***

The evaluation of a child with AAC begins with a rapid assessment of the airway, breathing, and circulation, with emergent stabilization as indicated. It then proceeds with a history and formal neurologic examination. A focused history includes the patient's affect and behavior leading up to the acute change, history of similar episodes, seizure activity, recent illness, fever or other infectious symptoms, recent medications, chemotherapy and radiotherapy, and status of the malignancy.

Clinical findings can localize the level of brain involvement. Altered breathing pattern, abnormal pupillary size and reactivity, abnormal extraocular movements, lack of spontaneous motor function, and reduced response to verbal or physical stimuli may indicate cerebral herniation. A diffuse increase in intracranial pressure may cause rostrocaudal or central herniation characterized by a symmetric downward displacement of the brain through the tentorium and gradual progressive failure of diencephalic and brainstem functions. In contrast, lateral mass lesions cause uncal herniation, shifting the brain and compressing the third nerve and lateral midbrain. Uncal herniation tends to be rapid and unpredictable and may culminate in a nonstereotypic brainstem herniation, with sparing of some brainstem functions (e.g., pupillary responses) despite compromise of lower brainstem functions (e.g., breathing). In the later stages of herniation, symptoms of the two forms often overlap. [89]

The causes of AAC can be focal or diffuse. Focal intracranial mass lesions that cause AAC with or without localized signs include primary CNS tumors, infarctions, hemorrhages, metastases, and abscesses. Acute thrombotic or, rarely, embolic CVAs can cause neurologic deterioration. [90] Subdural hematomas and intracranial hemorrhages may complicate coagulopathy, profound thrombocytopenia (usually fewer than 10,000 platelets per microliter), or L-asparaginase therapy.[90] Spontaneous hemorrhage into intracranial tumors, especially medulloblastomas and high-grade gliomas, or into metastatic tumors may occur with normal platelet counts and normal coagulation. Metastatic spread of tumor to the CNS occurs late in the disease in children with sarcomas, lymphomas, and primary renal tumors. Children with cancer who are immunosuppressed are prone to fungal or bacterial brain abscesses, meningitis, and sinusitis (see Chapter 41).

Diffuse disorders causing up to one-fourth of encephalopathy cases and mental status changes in children with cancer include metabolic derangements, drug toxicity, and infectious encephalitis.[91] Metabolic derangements that alter consciousness include anoxia, ischemia, uremia, hepatic failure, and electrolyte abnormalities. Metabolic disturbances infrequently lead to increased pressure and central herniation. Viral encephalitis, most commonly from varicella or herpes simplex, may result in acute coma. Seizures cause more than 50% of acute mental status changes in young patients; therefore, the postictal state must be differentiated from other changes in consciousness. Leukemic meningitis and hyperleukocytosis are relatively infrequent causes of AAC.

Table 39-4 lists the commonly used chemotherapeutic and supportive care agents that acutely alter a person's level of consciousness. Corticosteroids may cause personality changes and, occasionally, hallucinations and psychosis. [92] Repetitive high doses of cytosine arabinoside may cause symptoms of cerebellar dysfunction, seizures, coma, or death during an infusion or days later. [93,94] Both high-doseparenteral methotrexate and intrathecal methotrexate can cause transient encephalopathy or seizures, believed to be secondary to a direct toxic effect on the brain. [95,96] High-dose methotrexate can also cause focal neurologic deficit, presumably due to acute intracellular metabolic derangements. [95,97] Ifosfamideis associated with acute somnolence, neurologic deterioration, and coma, especially after prior cisplatin exposure or in patients with renal insufficiency. [98,99] The introduction of new drugs, use of higher doses of standard drugs, and the wider use of means to overcome the blood–brain barrier (e.g., intra-arterial infusion) are introducing more acute neurotoxicity. Intra-arterial infusions of bischloroethylnitrosourea (BCNU, carmustine) and cisplatin have been related to acute neurologic deterioration, seizures, strokes, and encephalopathy. [100,101] AAC may occur in patients with a familial deficiency of dihydropyrimidine dehydrogenase who are receiving 5-fluorouracil. [102] Thiotepaand BCNU, given in high doses in bone marrow transplant regimens, can result in severe, life-threatening encephalopathy. [100,101] Retinoids such as ATRA and fenretinide may lead to pseudotumor cerebri, a complication especially common in children.[32]

Neurotoxic agents prime the CNS for neurologic compromise. In this setting, relatively mild metabolic derangements or sedative drugs may suppress consciousness seemingly disproportionate to the insult. High-dose narcotics can sedate profoundly, and their rapid termination can lead to withdrawal symptoms. Benzodiazepines, widely used for conscious sedation, may acutely cause dysphoria. Poor excretion of narcotics due to hepatic dysfunction or overdoses due to malfunction of patient-controlled analgesia devices can lead to toxic drug levels. Transdermal fentanyl or scopolamine patches can cause sedation and, when placed behind the ear, ipsilateral pupillary constriction.

Up to one-half of patients receiving high-dose interleukin-2 experience disorientation and confusion; less common are paranoia and combativeness, and somnolence and coma are rare.[103] The interferons can cause subacute somnolence, altered cognition, psychiatric symptoms, conceptual disorganization, focal neurologic deficits, cortical blindness, coma, and death.[103]

***Therapy and Recommendations***

First, life-threatening respiratory and circulatory disturbances must be corrected. Next, a CBC, serum glucose, electrolytes, and ammonia level, hepatic and renal function tests, blood cultures, and a coagulation profile should be obtained. Symptoms or signs of increased intracranial pressure should be managed with intravenous dexamethasone (first dose, 1 to 2 mg per kg) and, possibly, infusion of mannitol in a 20% solution at 0.25 to 2.00 g per kg and, finally, if necessary, rapid-sequence intubation and hyperventilation. [89] Hyperventilation sufficient to decrease the partial pressure of blood carbon dioxide to 20 to 25 mm Hg reduces cerebral blood flow, but excessive hyperventilation may result in ischemia of the brain. [89] When stabilized,patients with presumed mass lesions or with unexplained coma should undergo emergent CT scanning or MRI with osmotic contrast agents only if there is no hyperviscosity or hyperosmolality. In patients with signs or symptoms of increased intracranial pressure in whom sepsis or meningitis is suspected, broad-spectrum antibiotics should be given prior to imaging. Neurosurgical intervention may be indicated for mass lesions or hydrocephalus unresponsive to medical management. Lumbar puncture should not be performed until a mass lesion has been ruled out. Thrombocytopenia and coagulopathy should be corrected before a lumbar puncture or surgery is attempted.

For chemotherapy-related neurologic alterations that threaten life or function, the medication should be discontinued or the dose reduced. The specific antidote for ifosfamide encephalopathy is methylene blue.[104] In the situation of high-dose cytosine arabinoside–induced neurologic dysfunction, the drug should be discontinued. Steroids may ameliorate the neurologic effects of interleukin-2 but may also interfere with the agent's efficacy. Acute CNS deterioration due to elevated methotrexate levels, stemming from either altered clearance or overdose, can be treated with leucovorin or carboxypeptidase (see the section Intrathecal Chemotherapy Overdoses or Errors). Acute methotrexate encephalopathy usually is reversible, and many patients tolerate subsequent use of methotrexate. Management of radiation-induced CNS necrosis is primarily supportive. When large areas of focal necrosis are present, surgical debulking may be helpful. [105] Although hyperbaric oxygenation and

anticoagulation have been recommended for the treatment of radiation-induced vasculitis, their efficacy is unproven. [106] In children with AAC who are receiving narcotics, 1 to 2 mg naloxone can be given intravenously, intramuscularly, or sublingually to reverse the sedation rapidly. The antidote for midazolam is flumazepine, 0.01 mg per kg initially, then 0.005 mg per kg every minute to a maximum dose of 1 mg.

## Cerebrovascular Accidents

### *Etiology*

In children with cancer, CVAs are due to cerebral arterial or venous thrombosis, intracerebral hemorrhage, local or metastatic spread of tumor, antineoplastic agents, hematologic abnormalities, or primary CNS infections; embolic causes are exceptional. [90] When a CVA occurs at the onset of illness, it usually is associated with disease-related coagulation abnormalities. During treatment, most strokes are related to a specific chemotherapeutic agent or infection. At the end stages of disease, sepsis, disseminated intravascular coagulation (DIC), CNS infection, and progressive tumor are common causes of CVA. Strokes occurring months to years after children have completed therapy generally are the result of radiation-induced vascular damage. [107]

### *Pathogenesis*

Patients with AML—especially APML, acute monoblastic leukemia, or any form of leukemia with hyperleukocytosis (see the section Hyperleukocytosis)—are at especially high risk for strokes at diagnosis or early in treatment. [108,109 and 110] Myelodysplasia or leukemia with peripheral hypereosinophilia and acute megakaryoblastic leukemia are associated with an increased risk of stroke. Leukemic promyelocytes enhance thrombin activation, and their high levels of expression of annexin II increase production of plasmin, a fibrinolytic protein, causing DIC and CVA. [108] The CVA usually occurs early in treatment of APML and may even be the presenting symptom.

L-Asparaginase as a single agent or in combination with vincristine and prednisone is associated with increased risk of venous thrombosis, especially near the end of induction therapy in ALL.[111] The majority of thromboses occur in the CNS.[112] The frequency of thrombotic complications in children with ALL varies from 2% to 12%. Children receiving L-asparaginase acquire a deficiency of antithrombin III but have enhanced thrombin formation, creating a prothrombotic state. [111] Intrathecal methotrexate occasionally causes CVA.[113]

During induction treatment for ALL or NHL, visual hallucinations progressing to confusion and stroke-like episodes may occur. They are associated with bilateral cortical or subcortical white-matter lesions. The etiology of these spells is believed to be vascular, possibly associated with hypertension or vincristine. [114] Transient ischemic events, similar to complicated migraine, may occur after cranial irradiation and chemotherapy. [115]

Radiotherapy may cause delayed large- and small-vessel occlusions. [107,116] Total doses of radiotherapy in excess of 5,000 cGy have been related to large-vessel occlusions. The peak incidence of large-vessel occlusions is 6 months to 3 years after treatment, but occlusions may take place up to two decades later. [116] Rarely, patients develop a CVA after lower doses or conventional fractions of radiation (180 to 200 cGy). More commonly, radiotherapy causes small, focal vascular occlusions. Mineralizing microangiopathy with dystrophic calcification is seen histopathologically in many children who have died of cancer but rarely is reported in children who have received less than 2,000 cGy. Intrathecal or parenteral methotrexate and cytosine arabinoside potentiate the risk of radiation-induced damage. [117]

Hemorrhagic CVA can occur preterminally, especially in children with neuroblastoma metastatic to the dura or torcula and in those with platelet-resistant thrombocytopenia.[91] Neuroblastoma may also cause lateral and transverse sinus thrombosis by compression from metastases to the calvarium that are adjacent to the venous channels.[91]

### *Evaluation and Differential Diagnosis*

In evaluating a child with cancer and a presumed CVA, it is important to consider the type, extent, and status of the cancer, the antineoplastic treatment, and any associated medical conditions. In a critically ill child with uncontrollable cancer, no specific evaluation may be indicated. CVAs usually present as acute impairments in motor function or speech, often with associated seizures. If symptoms do not clear within 24 hours after the ictus, a structural CNS lesion must be ruled out. A major CVA, such as a sagittal sinus thrombosis or a brainstem stroke, can cause obtundation, which must be differentiated from lethargy or coma.

When the child is medically stable, emergent CT or MRI should be performed with and without contrast medium. MRI often is more specific, as patients with small subcortical or small-vessel infarcts may have normal CT scans. Magnetic resonance angiography can help to confirm a specific diagnosis, such as postradiation vessel-occlusive vasculopathy,[107] but is rarely needed at the onset of symptoms. Follow-up imaging studies 7 to 10 days later may document infarction and demonstrate the full extent of damage.[118] Care should be taken to evaluate the torcular region of the calvarium and dura in children with neuroblastoma, to rule out the possibility of a sinus thrombosis. [91] If the CT or MRI scan does not show a focal mass lesion, lumbar puncture can be performed for analysis of opening pressure, protein and glucose levels, cell count, cytology, and bacterial, viral, and fungal cultures.

### *Therapy and Recommendations*

The management of a child with a CVA is primarily supportive. If there is evidence of DIC, supportive care includes platelet and fresh frozen plasma transfusions. The use of heparin is controversial. Heparin may be useful in patients whose CVA is thromboembolic but is contraindicated in hemorrhagic CVA. [13] The platelet count in children with hemorrhagic strokes should be kept above 75,000 per microliter to prevent further bleeding. In patients with DIC secondary to sepsis and low antithrombin III levels, replacement of antithrombin III in high doses tends to improve overall survival. [119] If CVA is a presenting or early symptom of malignancy, treating the underlying disease with cytotoxic therapy may prevent additional CVAs. Antibiotics or antifungal agents should be started if there is concern of infection.

The use of ATRA to treat APML often resolves the associated coagulopathy in 5 to 7 days [108]; however, many CVAs occur earlier. ATRA is believed to reverse the annexin II–mediated fibrinolysis by blocking transcription of the annexin II gene in t(15,17)-positive cells. [108] In patients with L-asparaginase–related CVA or coagulation abnormalities, prophylactic treatment with fresh frozen plasma (10 mL per kg) has been investigated, but it does not affect the antithrombin III deficiency.[111] Use of the newer antithrombin III concentrates is controversial.

In children with neuroblastoma metastatic to the torcular region, emergency irradiation of the sinus area can resolve symptoms rapidly. [91] Treatment of nonmetastatic sagittal sinus thrombosis with no evidence of hypernatremia includes corticosteroids and hyperosmolar agents to decrease the intracranial pressure. Anticoagulants may cause extension of a venous infarct.

In the child with an intracerebral hemorrhage, surgery may be lifesaving, but operative intervention may be unwarranted in patients with uncontrolled coagulopathy or in those at the end stage of their disease.

## Seizures

### *Etiology and Pathogenesis*

Seizures are transient, involuntary alterations of consciousness, behavior, motor function, sensation, or autonomic function due to excessive rate and hypersynchrony of neuronal discharges. Table 39-5 lists common causes of seizures in children with cancer. Seizures account for up to 60% of neurologic consultations on pediatric oncology services.[91,120] They are primarily due to the underlying malignancy, especially primary CNS tumors, tumors metastatic to the CNS, and meningeal leukemia, and to antineoplastic therapy. Intrathecal cytarabine and intrathecal and high-dose intravenous methotrexate can cause seizures, especially in children who have received cranial irradiation. [91,96] Whether vincristine directly causes seizures or whether vincristine-related seizures are due to the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) and hyponatremia remains unclear. Radiation-induced small-vessel disease, cerebral necrosis, and leukoencephalopathy provoke seizures.[105] CVAs, CNS infections, coagulopathy, and metabolic derangements also cause seizures. [91]

| |
|---|
| Tumor |
| Primary central nervous system tumor |
| Metastatic tumor |
| Leukemic meningitis |
| Hyperleukocytosis |
| Central nervous system infection |
| Viral |
| Bacterial |
| Fungal |
| Protozoal |
| Cerebrovascular accident |
| Treatment |
| Intrathecal methotrexate |
| Intrathecal cytosine arabinoside |
| L-Asparaginase |
| Metabolic abnormality |
| Hypoxia |

**TABLE 39-5. ETIOLOGY OF SEIZURES IN CHILDREN WITH CANCER**

### *Evaluation and Differential Diagnosis*

A seizure is a symptom of an underlying pathologic process. The investigation of the seizure is similar to that previously discussed for AAC and CVA and should take place as soon as the seizure is controlled. A history of any previous seizures, family history of seizures, and a list of medications and cancer chemotherapy or radiotherapy should be obtained. A CBC, serum electrolyte levels (including magnesium, calcium, and phosphorus), renal and hepatic functions, and coagulation studies should be checked. Emergent CT or MRI with and without contrast is indicated to look for hemorrhage or a mass lesion. On T2-weighted images, methotrexate-induced encephalopathy and seizures may appear as diffuse hyperintensities in the white matter and cerebral subcortical calcifications. [96] If, on CT or MRI, an obstructing mass lesion is ruled out, cerebrospinal fluid (CSF) should be obtained for cell counts, glucose, and protein, as well as cytology, bacterial and viral culture, and viral titers as clinically indicated. Electroencephalography can localize abnormal electrical activity in the brain.

### *Therapy and Recommendations*

Most seizures are self-limited; a prolonged seizure requires emergent management. [121] Adequate ventilation and circulation must be secured and metabolic abnormalities corrected, followed by the initiation of anticonvulsants. Table 39-6 lists recommended anticonvulsants and their doses. Either of the benzodiazepines, lorazepam or diazepam, is the initial therapeutic agent because of the rapid onset of action. [121] For treatment of prolonged or multiple seizures, phenytoin is added as it is not as sedating as are the barbiturates. Valproic acid and carbamazepine should be avoided owing to their potential marrow suppression. Most children with cancer and seizures receive antiepileptic medication while evaluation is under way. Those without markedly abnormal electroencephalograms and normal neuroimaging usually can discontinue therapy without a recurrence. Children whose first seizure is prolonged or who have repetitive seizures experience an increased incidence of seizure recurrence and generally require prolonged therapy with anticonvulsants. [91]

| Drug | Dose (mg/kg) | Duration of action | Advantages | Disadvantages |
|---|---|---|---|---|
| Diazepam | i.v.: 0.2–0.5 (max. 10 mg)<br>p.r.: 0.2–0.5 (max. 20 mg) | i.v.: 20–30 min<br>p.r.: up to 4 h | Rapid onset, rectal formulation | Short duration, respiratory depression, hypotension |
| Lorazepam | i.v.: 0.05–0.2 mg/kg/dose | 12–24 h | Rapid onset, longer duration of action | Respiratory depression, hypotension, drowsiness |
| Phenytoin | i.v.: 18 to 20 mg/kg bolus at 1–3 mg/kg/min | Up to 12 h | Rapid onset, minimal sedation | Arrhythmia, wide QT interval |
| Phenobarbital | i.v.: 18 to 20 mg/kg bolus at 1 mg/kg/min (max. 30 mg/kg) | Up to 24 h | Long half-life | Sedation, respiratory depression, bradycardia |
| Fosphenytoin sodium | i.m.: 10–20 mg PE/kg, 3 mg/kg/min (max. 150 mg/min) | Up to 12 h | Intramuscular formulation | Emesis, ataxia, rash, pruritus |

PE, phenytoin equivalent.
Adapted from: Bleyer WA. The acute management of seizures. Pediatr Ann 1990;19:8:215–221, with permission.

**TABLE 39-6. ANTICONVULSANTS FOR STATUS EPILEPTICUS IN CHILDREN WITH CANCER**

Antibiotics are indicated in febrile, neutropenic patients, those with meningeal signs or symptoms, and those in whom infection is a concern. In the case of CNS infection, metabolic abnormalities, and most therapy-related seizures, antiepileptics can be withdrawn after correction of the underlying abnormality if the follow-up electroencephalogram shows no epileptiform activity and no residual focal CNS defect is apparent.

### Intrathecal Chemotherapy Overdoses or Errors

Intrathecal (i.t.) chemotherapy is used frequently in the treatment of ALL, AML, and NHL and, unfortunately, overdoses and errors in administration occur. The symptoms noted with i.t. methotrexate overdoses range from none to headache for doses of less than 100 mg to seizure and coma within hours for doses in excess of 500 mg.[122] The only reported overdose of i.t. cytarabine (200 mg) was associated with dilated pupils for an hour. [123] Two inadvertent instillations of i.t. anthracyclines were notable for their delayed onset of neurologic symptomatology (16 and 17 days); one ended in death. [124] Infusion of i.t. vincristine, a CNS neurotoxin, causes a rapidly ascending paralysis and coma.[125] Of note, infusions of more than 500 mg of i.t. methotrexate and mistaken i.t. administration of vincristine at any dose usually are fatal.[124]

Immediate action is necessary for any chance of complete recovery after an i.t. overdose or error. If the interval between the overdose and recognition of the problem is less than 2 hours, as much CSF as possible should be drained. [122] Simple CSF drainage within 1 to 2 hours of infusion can allow the recovery of 30% to 50% of the initial drug dose.[124,126,127] If more time has elapsed, CSF can be exchanged with saline or Ringer's lactate solution. Ventricular catheter placement for ventriculolumbar perfusion should be considered if the patient's clinical condition continues to deteriorate or if the overdose is severe.

Overdoses of less than 100 mg methotrexate require minimal intervention; doses of greater than 500 mg usually are fatal, and doses between these extremes need aggressive therapy as outlined previously. [122] With the advent of i.t. carboxypeptidase $G_2$ ($CPDG_2$), survival without sequelae has occurred after i.t. methotrexate doses of more than 500 mg, even when discovered 2 hours after the initial instillation. [127,128] $CPDG_2$ is a member of a class of enzymes that hydrolyzes the C-terminal glutamate residue from folic acid and classic antifolates such as methotrexate, thereby converting methotrexate rapidly to a nontoxic metabolite. A pediatric study of the efficacy of $CPDG_2$ for the management of i.t. methotrexate overdoses in excess of 100 mg recommends immediate drainage of CSF by gravity for 5 minutes, followed by intrathecal administration of 2,000 units (two vials) of $CPDG_2$. High-dose systemic leucovorin (100 mg every 6 hours for four doses) and intravenous dexamethasone (0.5 mg per kg) every 6 hours for 24 to 72 hours should be administered concomitantly to decrease the severity of the chemical meningitis. [129] CNS drainage and systemic leucovorin usually suffice for i.t. methotrexate doses of less than 100 mg. [127] i.t. Leucovorin has been associated with worsening neurotoxicity [127] and should not be administered.

Only one of eight reported children survived accidental administration of i.t. vincristine. [124,125] CSF was drained from the surviving child within 5 minutes of the drug's administration, followed by three CSF exchanges with Ringer's lactate solution and fresh frozen plasma and 18 hours of ventriculolumbar perfusion with these same substances. The child also received glutamic acid (10 g intravenously over 24 hours, then 250 mg orally every 8 hours), leucovorin (25 mg intravenously every 6 hours for 1 week), and pyroxidine (50 mg intravenously every 8 hours for 1 week).[125,126] Of note, the only adult reported in the literature to have survived i.t. vincristine also underwent CSF exchange with Ringer's lactate solution and ventriculolumbar perfusion with Ringer's lactate solution and fresh frozen plasma. [130]

Intravenous corticosteroids have been used in several i.t. chemotherapy errors, as they theoretically decrease meningeal inflammation. However, insufficient data are

available to permit evaluation of their efficacy. i.t. Administration of corticosteroids is contraindicated because one must drain and exchange CSF immediately.

## Spinal Cord Compression

### *Etiology and Pathogenesis*

A mass that compromises the integrity of the spinal cord, conus medullaris, or cauda equina requires urgent attention to minimize long-term neurologic dysfunction. Epidural compression may occur with a known malignancy or may be the presenting symptom of an undiagnosed malignancy. Acute compression of the spinal cord occurs in 3% to 5% of children with cancer, often at diagnosis. [131,132] Another 5% to 10% of patients develop back pain that must be differentiated from spinal cord compression. Table 39-7 shows the frequency of epidural metastases among 2,259 children with malignant solid tumors at St. Jude Children's Research Hospital from 1962 to 1987.[132] Sarcomas account for most spinal cord metastases, whereas neuroblastoma, germ cell tumors, lymphoma, and dropped metastases from primary CNS tumors account for most of the remainder.[132] Spinal cord compression can occur with almost any tumor type, including Wilms' tumor [133] and leukemia.[134]

| Pathology | No. of cases of SCC (%) | Total cases |
|---|---|---|
| Ewing's sarcoma | 30 (17.9) | 168 |
| Neuroblastoma | 32 (7.9) | 402 |
| Osteogenic sarcoma | 16 (6.5) | 243 |
| Rhabdomyosarcoma | 14 (4.9) | 287 |
| Hodgkin's disease | 8 (2.0) | 404 |
| Soft tissue sarcoma | 4 (3.9) | 102 |
| Germ cell tumor | 5 (3.8) | 130 |
| Wilms' tumor | 2 (0.7) | 290 |
| Hepatoma | 1 (1.4) | 69 |
| Other* | — | 164 |
| Total | 113 (5.0) | 2,259 |

SCC, spinal cord compression due to epidural metastatic disease.
*Patients with other tumors who did not develop epidural spinal metastases.
Reproduced from Klein SL, Sanford RA, Muhlbauer MS. Pediatric spinal epidural metastases. J Neurosurg 1991;74:70, with permission.

**TABLE 39-7. INCIDENCE OF SPINAL CORD COMPRESSION IN CHILDREN WITH SOLID MALIGNANT TUMORS**

The spinal cord and cauda equina may be compressed by tumor in the epidural or subarachnoid space or, less commonly, by metastatic spread to the cord parenchyma. Epidural compression occurs by extension of a paravertebral tumor through the intervertebral foramina. Compression of the vertebral venous plexus by the tumor causes vasogenic cord edema, venous hemorrhage, and ischemia.[135] Metastatic involvement of the vertebral bodies and secondary compression of the spinal cord, which is common in adults with cancer, are rare in childhood. [136] Treatment-related myelitis may mimic cord compression.[136]

### *Evaluation and Differential Diagnosis*

Children with cancer and back pain should be considered to have spinal cord compression until proven otherwise. Local or radicular back pain occurs in 80% of children with cord compression.[131] Pain can start weeks to months before diagnosis. Long-standing cord compression can present as progressive weakness, sensory abnormalities, and paresis.[137] Once neurologic abnormalities are apparent, paraplegia and quadriplegia can occur rapidly and may be irreversible. [137] Compression at the level of the cauda equina may present with urinary and fecal incontinence.

Detailed neurologic examination, with attention given to extremity strength, reflexes, and determination of a sensory level, is essential. A rectal examination assesses sphincter tone. Localized tenderness to vertebral percussion occurs in many patients, and the level of maximal spinal tenderness is a reliable localizing sign. Most patients have objective loss of motor strength in the extremities at the time of diagnosis. Based on clinical findings, the level of spinal cord involvement can usually be determined (Table 39-8), but the absence of weakness or sensory abnormalities does not exclude spinal cord compression. History and physical examination can usually rule out vincristine neuropathy as the cause of back pain.

| | Location | | |
|---|---|---|---|
| Sign | Spinal cord | Conus medullaris | Cauda equina |
| Weakness | Symmetric, profound | Symmetric, variable | Asymmetric, may be mild |
| Tendon reflexes | Increased or absent | Increased knee, decreased ankle | Decreased, asymmetric |
| Babinski | Extensor | Extensor | Plantar |
| Sensory | Symmetric, sensory level | Symmetric, saddle | Asymmetric, radicular |
| Sphincter abnormality | Spared until late | Early involvement | May be spared |
| Progression | Rapid | Variable, may be rapid | Variable, may be rapid |

**TABLE 39-8. CLINICAL LOCALIZATION OF EPIDURAL CORD COMPRESSION**

Traditionally, spine radiographs have been the first radiologic study performed, owing to ease of performance and availability; however, abnormalities are revealed by this method in fewer than half the affected children. Historically bone scans, lumbar myelography, and metrizamide CT were the standard diagnostic tests. They have been supplanted by T1- and T2-weighted MRI scans, performed with and without gadolinium enhancement. [118,138] MRI demonstrates epidural disease, intraparenchymal spread of tumor, and small lesions compressing nerve roots in the cauda equina. [118,137,138] Any child who is not ambulatory at the time of clinical presentation, independent of the duration of dysfunction before evaluation, should undergo imaging immediately. In those children with localizing back pain and no focal findings on neurologic examination, MRI can be arranged within 24 hours. If MRI is not available, CT myelography should be performed. [135] CSF laboratory studies are essential in the evaluation of subarachnoid disease and meningeal leukemia or carcinomatosis. CSF protein concentration is elevated in patients with complete spinal cord block, but protein may be normal in patients with partial obstruction.

### *Therapy and Recommendations*

If the history suggests rapidly progressive spinal cord dysfunction or physical examination documents an anatomic level of dysfunction, the recommended approach is dexamethasone in a bolus dose of 1.0 to 2.0 mg per kg, followed by MRI investigation.[136,137] For the child with cancer and back pain in whom cord compression is possible but in whom loss of function and rapid neurologic progression of symptoms are not apparent, the situation is a subacute emergency and a lower dose of dexamethasone (0.25 to 0.50 mg per kg orally every 6 hours) suffices. Loblaw and Laperriere [139] have found evidence to support the use of high-dose dexamethasone (96 mg per day) in adults but little evidence supporting the use of moderate-dose dexamethasone (16 mg per day) in conjunction with radiotherapy.

If an epidural mass is compressing the spinal cord, the cord must be decompressed immediately. Although it reduces vasogenic cord edema and often results in neurologic improvement, dexamethasone is not an alternative to definitive spinal decompression. Local radiotherapy, surgical decompression, and chemotherapy all have their advocates. However, no randomized, controlled studies or prospective studies compare short- and long-term outcomes. A clear indication for surgery is the unknown primary tumor. For the child with epidural disease without known dissemination, surgery offers the dual benefit of decompression plus identification of the tumor type. Because pediatric tumors frequently enter the spinal canal by way of the intervertebral foramina, surgery involves laminectomy and posterior decompression.[137] Resection of multiple vertebral lamina in an infant or young child causes problems of growth and spinal stability. For the rare child or adolescent with spinal cord compression from vertebral body metastases or a radioresistant tumor, vertebral body resection may be needed. Surgery is indicated also if the

symptoms progress despite radiotherapy.[139]

If the diagnosis is known and the tumor is radioresponsive, radiotherapy is often the treatment of choice. The portal should include the full volume implicated on radiographic study plus a margin, depending on disease and patient-related factors. Supervoltage techniques are used, and daily doses between 180 and 400 cGy are given concomitantly with dexamethasone. Total dose depends on tumor histology and response to initial therapy. In adults, ambulatory radiotherapy and surgery offer similar outcomes, but radiotherapy is associated with decreased morbidity and mortality. [139] In an infant or young child, high-dose, wide-field radiotherapy will impair growth of the spine, spinal cord, and surrounding tissues.

A third alternative, chemotherapy, may be appropriate for patients with spinal cord compression due to lymphoma, leukemia, and neuroblastoma. [134,140] High-dose dexamethasone and systemic chemotherapy often result in prompt symptomatic improvement and reduction in mass size.[134] Ultimately, the optimal treatment often involves a combination of radiotherapy, surgery, and chemotherapy, planned by a multidisciplinary team.

The prognosis for patients with spinal cord compression depends on the neurologic findings at presentation. Patients who are ambulatory when treatment is started remain ambulatory.[137] In adults, patients who are paraplegic at the time of treatment rarely regain function. [139] However, in a recent series, one-half of the children who were not ambulatory at the beginning of treatment regained the ability to walk after emergency treatment. [131]

### Hyperleukocytosis

#### *Etiology*

Hyperleukocytosis is defined as a peripheral leukocyte count exceeding 100,000 per microliter, but clinically significant hyperleukocytosis occurs with WBC counts of more than 200,000 per microliter in AML and in excess of 300,000 per microliter in ALL and chronic myelogenous leukemia (CML). Hyperleukocytosis occurs in 9% to 13% of children with ALL, 5% to 22% of children with AML, and almost all children with CML in the chronic phase. [141,142 and 143] Hyperleukocytosisis more common in infant ALL, AML, T-cell ALL with a mediastinal mass, and hypodiploid ALL. [141]

Hyperleukocytosis can cause death by CNS hemorrhage or thrombosis, pulmonary leukostasis, and the metabolic derangements that accompany tumor lysis. Table 39-9 classifies complications experienced by 234 children with acute leukemia and hyperleukocytosis. Of the 73 patients with AML, 23% died during early induction from pulmonary leukostasis or intracerebral hemorrhage. In contrast, 5% of 161 patients with ALL died of complications of TLS (see the section Metabolic Emergencies).[142]

| Complications | ALL (N = 161) | ANLL (N = 73) | p Value* |
|---|---|---|---|
| Metabolic† | 22 | 4 | .09 |
| Hyperkalemia | 16 | 2 | |
| Decreased calcium, increased phosphorus | 15 | 3 | |
| Acute renal failure | 5 | 4 | |
| Respiratory | 0 | 6 | <.001 |
| Hemorrhagic | 4 | 14 | <.001 |
| Central nervous system | 2 | 9 | |
| Gastrointestinal | 0 | 2 | |
| Pulmonary | 2 | 3 | |
| Pericardial | 0 | 1 | |
| Death | 8 | 17 | <.001 |

ALL, acute lymphocytic leukemia; ANLL, acute nonlymphocytic leukemia.
*For comparison of frequencies of the indicated complication in patients with ALL versus those with ANLL.
†Some patients experienced more than one complication.
From Bunin NJ, Pui CH. Differing complications of hyperleukocytosis in children with acute lymphoblastic or acute nonlymphoblastic leukemia. J Clin Oncol 1985; 3:1590, with permission. Copyright 1985, Grune & Stratton, Inc.

**TABLE 39-9. EARLY COMPLICATIONS IN PATIENTS WITH HYPERLEUKOCYTOSIS**

#### *Pathogenesis*

Hyperleukocytosis directly increases blood viscosity by increasing the packed leukocyte volume. It indirectly increases blood viscosity by the formation of leukemic cell aggregates and thrombi.[144] Leukemic blasts are not easily deformed and tend to trap plasma between them. As myeloblasts are larger (350 to 450 $mm^3$) than lymphoblasts (250 to 350 $mm^3$), the former cause greater increases in viscosity, and myeloblasts are also more likely to aggregate owing to their "stickier" nature. [142,144] At very high WBC counts, leukemic aggregates proliferate within the cerebral vasculature and in the brain itself, resulting in damage to vessels and hemorrhage. [145] Tryka et al.[146] characterized the pathology of pulmonary leukostasis as "leukemic cell lysis pneumonopathy," in which degenerating aggregates of blasts in the vessels and interstitium release their intracellular contents, damaging alveoli diffusely. Leukemia-associated respiratory failure is exacerbated by pulmonary hemorrhage and possible toxins released from the blast cells that damage pulmonary endothelium. [147]

#### *Evaluation and Differential Diagnosis*

Frequently, a CBC revealing the significantly elevated WBC is obtained prior to arrival at a tertiary care center. The child with a WBC exceeding 100,000 per cubic milliliter should be evaluated for signs and symptoms of hyperleukocytosis. Many will be asymptomatic, but others will present with mental status changes, headaches, blurred vision, seizures, coma, and symptoms of stroke, papilledema, and retinal artery or retinal vein distention, all attributable to the hyperviscosity in the cerebral vessels.[142,144] Pulmonary leukostasis causes dyspnea, hypoxia, acidosis, and cyanosis. Priapism, clitoral engorgement, and dactylitis have been described with hyperleukocytosis.[148,149] Additional laboratory studies include serum electrolytes, uric acid, renal function tests, and a coagulation profile. A chest radiograph may reveal a mediastinal mass or diffuse interstitial infiltrates.

#### *Therapy and Recommendations*

No controlled studies are available on the management of hyperleukocytosis. As outlined in the section Tumor Lysis Syndrome, intravenous hydration at two to four times the maintenance volume, alkalinization with sodium bicarbonate, and allopurinol should be started. [150] Patients with platelet counts of fewer than 20,000 per microliter should receive platelet transfusions to prevent cerebral hemorrhage, as platelets do not add substantially to blood viscosity. In contrast, packed red cells increase viscosity.[151] The hemoglobin level should not be raised above 10 g per dL; most children are asymptomatic with hemoglobins of 7g per dL. Exchange transfusion or leukapheresis can rapidly lower the WBC count and may improve coagulopathy. [148] Pediatric studies found a 52% to 66% mean reduction in WBCs with exchange transfusion and a 48% to 62% reduction with leukapheresis. [141,152] Maurer et al.[153] noted a significantly lower incidence of severe TLS in patients with ALL (WBCs in excess of 200,000 per $mL^3$) who underwent leukapheresis as compared to those who did not. Neurologic abnormalities, respiratory distress, and priapism have improved after leukapheresis in patients with AML, ALL, and CML. [148] Whether leukapheresis reduces the risk of CNS hemorrhage in AML is unknown. [148] Problems associated with leukapheresis are the need for anticoagulation, difficulty with access in small children, and limited availability in many hospitals. Exchange transfusion and leukapheresis are only temporizing. Systemic antileukemic therapy must be initiated as soon as problems such as SVCS, SMS, and compromised renal function have been addressed.

Cranial irradiation to a dose of 400 cGy has been administered to prevent CNS hemorrhage. However, the risk of CNS hemorrhage in ALL is small: No cases occurred in 136 patients with counts between 100,000 and 400,000 per microliter. [142] Most pediatric oncologists do not use prophylactic CNS irradiation. [148,152]

## METABOLIC EMERGENCIES

### Tumor Lysis Syndrome

TLS consists of the metabolic abnormalities that result from the death of tumor cells and release of their contents into the circulation. The classic triad of TLS includes

hyperuricemia, hyperphosphatemia, and hyperkalemia. Symptomatic hypocalcemia can develop secondary to formation of calcium phosphate from the hyperphosphatemia. Although TLS can occur before any cytotoxic therapy, its manifestations usually appear 12 to 72 hours from the initiation of therapy.

### *Etiology and Pathophysiology*

TLS occurs in patients with tumors that have a high growth fraction, large volume, or wide dissemination and that are sensitive to cytotoxic therapy. It occurs most commonly in Burkitt's lymphoma, lymphoblastic lymphoma, and ALL, particularly T-cell–lineage ALL with hyperleukocytosis and extensive extramedullary disease. TLS has also been noted in neuroblastoma, medulloblastoma, breast carcinoma, and small cell lung carcinoma.[154] TLS is rare in AML and CML, despite high WBC counts. The literature contains descriptions of children who present with hyperuricemia and acute renal failure as the initial manifestation of occult lymphoproliferative malignancy.[154,155 and 156] Among 37 patients with Burkitt's lymphoma, Cohen et al.[157] determined that bulky abdominal tumors, elevated pretreatment serum uric acid and lactate dehydrogenase concentrations, poor urine output, or low glomerular filtration rate predisposed a patient to severe metabolic derangements. Andreoli et al.[158] found that advanced age (10.4 ± 5.4 years) correlated with development of renal failure in children with ALL. This may, in part, be due to the progressive decline in the fractional excretion and clearance of uric acid that accompanies advancing age. These researchers did not find a high WBC to be a predictor of renal failure.[158]

TLS is a direct result of the release into the circulation of the nuclear and cytoplasmic degradation product of malignant cells. Potassium, the principal intracellular cation, increases in the serum, and its excretion is reduced in renal insufficiency. A rapid rise in potassium can cause cardiac arrest in minutes or hours. Elevated uric acid comes from the breakdown of the released nucleic acids. In the presence of hyperuricemia, renal excretion of uric acid initially increases but then decreases as uric acid crystals precipitate in the collecting ducts of the renal tubules owing to the acid environment of the kidney.[155] Lymphoblasts are especially rich in phosphate, having four times the content of normal lymphocytes.[159] Elevated levels of serum phosphate are exacerbated by a metabolic acidosis, which induces a shift of intracellular phosphate into the extracellular space.[155] When the solubility product factor (Ca × P) reaches 60, calcium phosphate precipitates in the microvasculature, causing a secondary hypocalcemia.[159] Precipitation of uric acid crystals and calcium phosphate within the renal tubules and microvasculature leads to acute renal failure.

### *Evaluation and Differential Diagnosis*

In a patient at risk for TLS, pertinent historic information includes the time of onset of symptoms referable to the malignancy. Symptoms can include abdominal pain or fullness, back pain, vomiting, diarrhea, dehydration, anorexia, cramps, spasms, tetany, seizure, and alterations in consciousness suggestive of hypocalcemia. On examination, special attention should be given to blood pressure, cardiac rate and rhythm, abdominal masses, presence of pleural effusions or ascites, signs of SVCS or SMS, and signs of cerebral anoxia.

Initial studies include a CBC, determination of serum sodium, potassium, chloride, bicarbonate, calcium, phosphorus, uric acid, BUN, and Cr levels, and urinalysis. If the serum calcium is low, an ionized calcium and serum albumin assessment should be obtained as well. An electrocardiogram is essential if the serum potassium level is greater than 6.0 mEq per L. It may show QRS widening and peaked T waves. When the leukocyte count is high, the serum potassium can be artifactually elevated by spontaneous lysis of leukocytes, platelets, and erythrocytes (i.e., pseudohyperkalemia).[160] In pseudohyperkalemia, no cardiac or electrocardiographic changes occur, and the actual potassium concentration can be determined from plasma rather than serum.[160] Hypocalcemia can cause a prolonged $QT_C$ interval on electrocardiography. Patients with electrocardiographic abnormalities should be placed on a cardiac monitor. Because acute renal insufficiency due to obstruction can have manifestations similar to those of TLS, US or CT should be performed on any child with an abdominal or pelvic mass, to rule out obstructive renal failure. Obstructive renal failure can be improved rapidly with urinary catheterization and is exacerbated by hydration.[81] A chest radiograph may reveal a mediastinal mass.

### *Therapy*

Early and aggressive intervention effectively reduces the morbidity associated with TLS. Patients with newly diagnosed leukemia or NHL should receive hydration, alkalinization, and allopurinol (Table 39-10). For most patients, this regimen suffices to prevent clinically significant tumor lysis and renal failure. When severe metabolic abnormalities have improved, cytotoxic therapy can commence.

| | |
|---|---|
| Hydration | $D_5{}^1/_2NS$ with 40 mEq/L $NaHCO_3$ – no potassium<br>2–4 times maintenance fluid rate<br>Maintain urine output at >100 mL/$m^2$ per h, urine specific gravity at <1.010 |
| Alkalinization | Maintain urine pH at 7.0–7.5; increase $NaHCO_3$ as needed<br>Stop $NaHCO_3$ if serum bicarbonate level reaches 30 mEq/L or the urine pH >7.5 |
| Diuresis | Avoid if hyponatremia present<br>Furosemide (0.5–1.0 mg/kg)<br>Mannitol (0.3 g/kg over 15 min) |
| Uric acid reduction | Start allopurinol (300 mg/$m^2$ per day or 10 mg/kg per day)<br>Urate oxidase i.v. if indicated |
| Metabolic abnormalities<br>Hyperkalemia | Monitor electrolytes, Ca, Mg, $PO_4$ q4–8h<br>Sodium polystyrene sulfonate (Kayexalate, 1 g/kg with 50% sorbitol)<br>Calcium gluconate (100–200 mg/kg)<br>Insulin (0.1 units/kg) plus 25% glucose (2 mL/kg) |
| Hyperphosphatemia<br>Hypocalcemia | Aluminum hydroxide (15 mL q4–8h)<br>Calcium gluconate i.v. only if symptomatic |
| Dialysis Indications | Volume overload: pleural, pericardial effusions<br>Renal failure<br>Hyperkalemia<br>Hyperphosphatemia<br>Hyperuricemia<br>Symptomatic hypocalcemia |

$D_5{}^1/_2NS$, 5% dextrose with 0.25% normal saline

**TABLE 39-10. MANAGEMENT OF PATIENTS AT RISK FOR TUMOR LYSIS SYNDROME**

Hydration is probably the most critical factor in treatment. Increased hydration translates to increased urinary outflow and an improved glomerular filtration rate. Patients should receive two to four times the maintenance fluid volume as 5% dextrose in 0.25% normal saline, with 40 to 80 mEq of sodium bicarbonate per liter, to produce a urine pH of 7.0 to 7.5. Urine output should be maintained at more than 100 mL per $m^2$ per hour, with a specific gravity of no more than 1.010. Potassium and calcium should not be added to hydration fluids unless a patient has symptomatic deficiencies. Although contraindicated in the patient with volume depletion, diuretics and mannitol may be indicated in patients with poor urine output because of accumulation of the infused fluid in the third space.[155] If urine output falls below 60 mL per $m^2$ per hour, mannitol can be given at 0.5 mg per kg over 15 minutes, followed by furosemide (0.5 to 1.0 mg per kg).

Allopurinol (250 to 500 mg per $m^2$ per day to a maximum of 800 mg) directly inhibits the formation of uric acid by blocking the enzyme xanthine oxidase, which converts hypoxanthine and xanthine to uric acid. An alternative widely used in Europe but still under investigation in the United States is urate oxidase (Uricase), which converts uric acid to allantoin and does not require alkalinization.[155]

Alkalinization of the urine aids in solubilizing uric acid. Sodium bicarbonate should be discontinued when plasma levels of uric acid normalize and cytotoxic therapy begins.[161] Overzealous alkalinization (urine pH in excess of 7.5) can lead to worsening nephropathy. At a pH above 7.5, xanthine and hypoxanthine stones may form and, at a pH of 8 or higher, calcium phosphate may crystallize in the kidneys.[161]

Patient weight should be measured once or twice daily. Serum electrolytes must be monitored; the frequency of obtaining them depends on the risk level of the patient. In a patient with disseminated Burkitt's lymphoma who presents with TLS, metabolic studies should be repeated 4 hours after initiation of therapy and monitored at least four times daily. Additional interventions should be started when metabolic abnormalities are worsening, in an attempt to avoid dialysis. Aluminum hydroxide, a phosphate binder (15 mL every 8 hours, escalated to a continuous nasogastric infusion as needed), will increase excretion of phosphate. Sodium polystyrene sulfonate (Kayexalate, 1 g per kg orally with 50% sorbitol), a potassium-binding resin, may help to lower a rising potassium level. Calcium gluconate (100 to 200 mg per kg per dose) can shift potassium intracellularly and stabilize myocardial conduction. Insulin (0.1 units per kilogram of rapid-acting insulin) with 2 mL per kg of 25% glucose in water as an intravenous bolus also promotes intracellular influx of potassium.[135] When hyperphosphatemia is present, treatment of hypocalcemia with intravenous infusions of calcium gluconate (100 to 2,000 mg per kg per dose) should be reserved for those individuals with signs and symptoms of hypocalcemia such as tetany, arrhythmias, or seizures. Increasing the serum calcium level will increase the calcium-phosphorus solubility factor product, favoring calcium phosphate deposition and renal failure.

When medical interventions fail to correct electrolyte disturbances or oliguria persists, dialysis may be necessary. Table 39-10 outlines the indications for starting

dialysis. Hemodialysis is preferable to peritoneal dialysis as it corrects electrolyte abnormalities more rapidly. Uric acid and phosphate levels fall as urine output increases.[155] Peritoneal dialysis is contraindicated with an abdominal or pelvic tumor. Continuous venovenous hemofiltration has been used prophylactically in patients with Burkitt's lymphoma to prevent renal failure,[162] but the benefits are unclear. Because tumor lysis often occurs in the setting of a high leukocyte count, some practitioners have advocated leukapheresis or exchange transfusion to reduce the tumor load and subsequent massive tumor lysis. These methods have not been subjected to any controlled analysis.

## Hypercalcemia

Hypercalcemia is defined as a serum calcium level in excess of 12 mg per dL. Levels higher than 12 mg per dL affect multiple organ systems, and levels exceeding 20 mg per dL can be fatal.

### *Etiology and Pathophysiology*

Over a span of 29 years at St. Jude Children's Research Hospital, hypercalcemia occurred in 25 children with cancer. [163] The 25 represented an incidence of 0.4%, much lower than the 5.0% to 20.0% noted in adults with cancer.[164] Of the 25 affected children, 10 had ALL and 4 had rhabdomyosarcoma.[163] Lymphomas, rhabdoid tumors, Ewing's sarcoma, neuroblastoma, brain tumors, hepatoblastoma, and ovarian carcinoma in teenagers may cause hypercalcemia. [163,165,166 and 167] As contrasted to patients with solid tumors and lymphomas who developed hypercalcemia later in their disease course and whose hypercalcemia proved to be more resistant to therapy, children with acute leukemia were more likely to present with hypercalcemia, and their hypercalcemia was more likely to respond to therapy. [163]

Malignant hypercalcemia can be caused by a defect in renal excretion, an increase in bone resorption, or a combination of both. Seymour and Gagel [168] classified malignant hypercalcemia into three categories: humoral, osteolytic, and calcitriol-mediated. Most common in adults is humoral hypercalcemia. The tumor produces an ectopic hormone that causes parathyroid hormone–like effects: increased osteoclastic bone resorption, increased renal resorption of calcium, and increased renal phosphate loss. Parathyroid hormone rarely is elevated in these patients, but a parathyroid hormone–related peptide has been found to be elevated in children with rhabdomyosarcoma and in women with breast cancer who have hypercalcemia.[169] $PGE_2$ in breast cancer and growth factors secreted by tumors can also cause bone resorption.[170]

In osteolytic hypercalcemia osteoclasts activated at the site of bone metastases resorb bone with the participation of various cytokines. Osteoclast-activating factor has been associated with hypercalcemia in patients with multiple myeloma and Burkitt's lymphoma. [171,172] Calcitriol-mediated hypercalcemia is most frequent in HD and NHL.[168] The calcitriol syndrome is characterized by increased intestinal absorption of calcium, increased renal excretion, and normal serum phosphate levels and metabolism.[168]

### *Evaluation and Differential Diagnosis*

Gastrointestinal, renal, neuromuscular, and cardiovascular symptoms dominate the clinical picture of malignant hypercalcemia ( Table 39-11). Early nonspecific symptoms include nausea, constipation, and polyuria, but increasing serum calcium levels lead to profound muscle weakness, renal insufficiency, bradyarrhythmias, and coma. Anorexia, vomiting, and polyuria initiate a self-sustaining spiral of dehydration, which leads to a decreased glomerular filtration rate and reduced renal calcium excretion. The early symptoms can mimic TLS in the patient with newly diagnosed leukemia, and the two disorders can overlap. Harguindey et al. [165] found that all seven of their patients with malignant hypercalcemia had elevated BUN and uric acid levels, normal or increased phosphorus concentration, and a metabolic alkalosis. Serum calcium, phosphate, BUN, Cr, uric acid, and ionized calcium levels should be determined. Serum levels of parathyroid hormone, parathyroid hormone–related peptide, and 25-(OH) vitamin D and 1,25-$(OH)_2$ may help to define the origin of the hypercalcemia. An electrocardiogram may reveal a prolonged PR interval with broad T waves. A bone scan or skeletal survey may show bony metastases.

| |
|---|
| **Gastrointestinal** |
| Anorexia |
| Nausea |
| Vomiting |
| Constipation |
| Ileus |
| **Neuromuscular** |
| Lethargy |
| Apathy |
| Depression |
| Fatigue |
| Hypotonia |
| Obtundation |
| Stupor |
| Coma |
| **Cardiovascular** |
| Bradycardia |
| Arrhythmia |
| Digitalis toxicity |
| **Renal** |
| Polyuria |
| Nocturia |

**TABLE 39-11. SIGNS AND SYMPTOMS OF HYPERCALCEMIA OF MALIGNANCY**

Medications that exacerbate hypercalcemia of malignancy include thiazide diuretics, oral contraceptives, tamoxifen, antacids with calcium carbonate, and lithium. Hypervitaminosis A or D, renal disease, granulomatous disease, adrenal insufficiency, fractures, and immobilization may also contribute.

### *Therapy*

Calcium levels exceeding 12.0 mg per dL require immediate correction. Treatment consists of four components: hydration, increased renal calcium excretion, decreased calcium mobilization from bone, and treatment of the underlying malignancy. For serum calcium levels of less than 14 mg per dL, hydration with furosemide diuresis may suffice.[158,173] With higher serum calcium levels, the recommended therapy is vigorous forced diuresis starting with normal saline repletion at two to three times maintenance volume. When a good urine output is obtained, furosemide (2 to 3 mg per kg every 2 hours) is started. [135] Furosemide blocks calcium resorption by the kidney and can decrease serum calcium by 3 mg per dL in 48 hours. Forced diuresis requires monitoring of both intravascular volume and serum and urine electrolytes; profound fluid shifts and potassium and magnesium losses may accompany sodium, calcium, and fluid excretion.

Prednisone, 1.5 to 2.0 mg per kg per day, slowly reduces the serum calcium level if hypercalcemia is mediated by osteoclast-activating factor, $PGE_2$, or calcitriol. Salmon calcitonin (4 MRC units per kilogram) acts within hours to reduce the serum calcium level by inhibiting bone resorption and promoting calcium excretion. Resistance to exogenous calcitonin develops within days, although the combined use of steroids and calcitonin may provide control for longer periods. Mithramycin, an antineoplastic antibiotic, lowers calcium within days but is too cytotoxic for prolonged use. [135]

Bisphosphonates inhibit osteoclast-mediated resorption of bone and reduce osteoclast viability. Bisphosphonates are highly effective in the treatment of hypercalcemia and have a long duration of action. Side effects include transient lymphopenia, fever, myalgia, gastrointestinal upset and, most seriously, prolonged hypocalcemia, hypophosphatemia, and hypomagnesemia. Although most data on the use of bisphosphonates for hypercalcemia involve adults, the literature on bisphosphonate use in the pediatric population is growing. Most pediatric data concern intravenous pamidronate. [174] Clinical response occurs in 12 to 48 hours after administration, and serum calcium levels usually normalize within 3 to 7 days. [174] Body et al.[175] determined that the proper starting dose in adults was 0.25 to 1.50 mg per kg. A recommended starting dose for children is 0.5 to 1.0 mg per kg infused over 4 to 6 hours, with close monitoring of serum calcium, phosphate, and magnesium levels.[174,176] A subsequent dose of 1 mg per kg can be given if necessary. Oral bisphosphonates are available, but pediatric experience with them is limited.

## Syndrome of Inappropriate Secretion of Antidiuretic Hormone (Hyponatremia)

Excessive secretion of antidiuretic hormone (ADH) accompanying a normal or low plasma osmolality or serum sodium concentration is termed *inappropriate* because it further depresses the levels of these chemicals. Symptoms of excessive ADH secretion are not usually apparent until the plasma sodium level falls to less than 120

mmol per L. A fall in the serum sodium level to less than 120 mmol per L within 24 hours or a gradual decrease in serum sodium to less than 115 mmol per L can be life threatening.

### *Etiology and Pathophysiology*

SIADH is characterized by the release of ADH without any relation to plasma osmolality. Excessive secretion of ADH increases water resorption by the kidneys and incites dilutional hyponatremia.[170] Hyponatremia associated with SIADH is associated with the use of vincristine, vinblastine, cyclophosphamide, ifosfamide, cisplatin, and melphalan.[177,178] Cyclophosphamideand ifosfamide are believed to reduce free-water clearance independent of ADH, and the resultant hyponatremia is aggravated by aggressive hydration, which is used to prevent cystitis related to cyclophosphamide and ifosfamide. [177]

SIADH can also occur in the setting of CNS injury or CNS disease; stress, pain, surgery, or positive-pressure ventilation; pulmonary infection and inflammation; and tumors, such as small cell lung carcinoma, lymphoma, or gastrointestinal carcinoma.[177] CNS disease stimulates release of ADH from the posterior pituitary.

### *Evaluation and Differential Diagnosis*

Most hyponatremia is asymptomatic and usually is diagnosed by routine laboratory studies. Early symptoms include fatigue, nausea, and anorexia; later manifestations are lethargy, confusion, seizures, and coma.[170] Although severe hyponatremia in children with cancer often is related to SIADH, the most common cause of mild hyponatremia is iatrogenic: simple overhydration with a hypotonic solution such as 5% dextrose in 0.25% normal saline. Hyponatremia can also be caused by failure to administer stress doses of glucocorticoids in a patient who has recently discontinued systemic steroids. Hypothyroidism, heart failure, acute renal failure, pancreatitis, and use of diuretics may exacerbate hyponatremia. Diabetes insipidus occurs in children with Langerhans' cell histiocytosis or with suprasellar tumors, either from the tumors or after irradiation. Diabetes insipidus usually presents with polydipsia, polyuria, and hypernatremic volume depletion. However, if the patient has been replacing losses with water or other hypotonic solutions, hyponatremia may develop. In patients with CNS tumors and renal damage, SIADH may need to be differentiated from cerebral salt wasting.[179,180]

The following studies should be obtained: renal and liver function tests, serum osmolality, and urinalysis, including a specific gravity, urine sodium and creatinine levels, and osmolality. The diagnosis of SIADH is made if the serum osmolality (usually less than 280 mmol per L) is lower than the urine osmolality (often greater than 500 mmol per L).

### *Therapy and Recommendations*

Fluid restriction is the mainstay of therapy for mild hyponatremia due to water intoxication, chronic SIADH, or acute SIADH if the sodium level exceeds 120 mmol per L and the patient is asymptomatic.[181,182] In cases of severe neurologic involvement (seizures, coma), boluses of 3% hypertonic saline to replace sodium losses should be started, followed by 1 mg of furosemide per kg to diurese free water. The rate of sodium correction should not exceed 2 mmol per L per hour, as too rapid a correction of sodium can cause cerebral edema, further neurologic deterioration, and death. [181,182] Urine output should be monitored closely, and frequent monitoring of serum electrolytes also is essential.

## Shock

Shock occurs when cardiovascular dysfunction results in inadequate perfusion of vital organs. Shock is common in children with cancer. The American Heart Association classifies shock according to either its etiology or its effects on blood pressure or cardiac output. [183]

### *Etiology and Pathophysiology*

The etiologic classifications are hypovolemic shock, cardiogenic shock, and distributive shock ( Table 39-12). Many entities fall into two categories. Hypovolemic shock is the most common type. In pediatric cancer patients, it usually is associated with bacterial sepsis. Hypovolemic shock may also occur with an addisonian crisis in patients who have received high doses of glucocorticoids in the previous 6 months and then have discontinued them. Pancreatitis may cause hypovolemic and distributive shock.

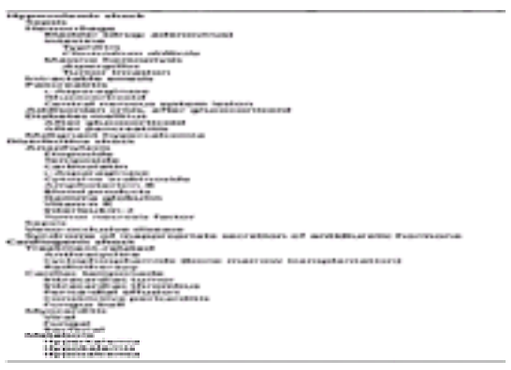

**TABLE 39-12. COMMON CAUSES OF SHOCK IN THE CHILD WITH CANCER**

Distributive shock occurs when there is inappropriate distribution of the blood volume. In cancer patients, the most common causes of distributive shock are drug-related anaphylaxis and sepsis (Table 39-12). Distributive shock occurs with high-dose boluses of interleukin-2. Delayed anaphylactic shock may take place weeks after administration of interleukin-2, when patients receive radioactive contrast. Hematopoietic growth factors and interferons have also been associated with anaphylactic reactions. Amphotericin-B causes hypotension and rigors, which, if uncontrolled, can lead to ventilation-perfusion defects, causing a patient in compensated shock to decompensate fully, with respiratory and cardiac failure.

Cardiogenic shock occurs in patients who have received moderate or high doses of anthracyclines with or without cardiac irradiation. It can occur after high-dose cyclophosphamide use during bone marrow transplant cytoreduction. A mass within the cardiac chambers or constrictive or effusive pericarditis can lead to cardiogenic shock.

Shock is a manifestation of inadequate organ perfusion secondary to inadequate cardiac output. [183] In hypovolemic shock, the cardiac output is low; in cardiogenic shock and in anaphylaxis, it may be high, with a mismatch between blood flow and organ needs. The body attempts to compensate for inadequate perfusion first by increasing heart rate and then by reducing perfusion to the kidneys, splanchnic bed, and skin. [183] Only when these mechanisms fail to compensate does hypotension ensue.

### *Evaluation and Differential Diagnosis*

The respiratory and cardiovascular systems must be assessed rapidly. Increased heart and respiratory rates; signs of respiratory distress (air hunger, nasal flaring, retractions, stridor, grunting, use of accessory muscles); weak peripheral pulses; pale, gray, or mottled skin; and cold extremities may be indications of impending cardiovascular collapse. Airway patency should be assessed, as masses can block the airway and cause shock. Capillary refill time should be used to assess peripheral perfusion. The status of the brain is best assessed by the level of consciousness, the patient's ability to respond to normal stimuli and to pain, the patient's generalized muscle tone and pupil size, and the presence of posturing or seizures.

### *Therapy*

For detailed management of shock, the reader is referred to the Textbook of Pediatric Emergency Medicine.[184] Therapies include establishing an airway and providing 100% oxygen by the least traumatic means possible. Initial fluid resuscitation should be 20 mL per kg of 0.9% normal saline or Ringer's lactate solution given as a rapid bolus over 10 to 20 minutes. In the absence of a response, this can be repeated up to 60 mL per kg or more in the first hour.[184] Concomitantly, the underlying etiology should be sought and treated. If the cause is presumed to be sepsis, cultures should be obtained and appropriate antibiotics started without delay. A chest radiograph is needed if the patient has respiratory symptoms. A CBC, electrolytes assessment, and tests of renal and hepatic function are necessary to determine proper replacement fluids. If there is evidence of hemorrhage, the patient should receive packed red cells (see Chapter 40), platelets, coagulation factors, and a surgical evaluation if there is a surgically correctable cause of hemorrhage.

In anaphylactic shock, the suspected drug should be discontinued immediately. Epinephrine 1:1,000 (0.01 mL per kg) should be given subcutaneously. If there is poor peripheral perfusion, epinephrine 1:10,000 (0.01 mL per kg) is given intravenously at a rate of 0.01 mg per kg over 1 to 2 minutes. Antihistamines—either diphenhydramine (1 to 2 mg per kg intravenously or intramuscularly) or hydroxyzine (0.5 to 1.0 mg per kg)—should be given. The use of steroids in anaphylaxis may prevent late-phase reactions. Steroids often are used for blood product reactions.

In cardiogenic shock, an electrocardiogram and an echocardiogram should be obtained immediately. An abnormal rhythm must be appropriately corrected. Use of adrenergic agents should be planned with the consultation of cardiologists and intensivists. If there is cardiac tamponade, the cause of the tamponade must be relieved surgically and emergently.

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# 40

# HEMATOLOGIC SUPPORTIVE CARE AND HEMATOPOIETIC CYTOKINES

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## INTRODUCTION

The success of more intensive treatment of children with cancer during the last two decades is associated directly with advances in supportive care. Hematopoietic cytokines, safer blood products, and a better understanding of the potential benefits and risks of their use are the focus of this chapter. The pathophysiology, differential diagnosis, and practical aspects of management of hematologic complications of childhood cancer are discussed, with emphasis on therapy, including transfusion and cytokine support.

## ANEMIA

### Pathophysiology

Anemia is the most commonly appreciated and readily managed of the hematologic complications of childhood cancer. The relatively long survival of erythrocytes (120 days) usually leads to a slow decline in hemoglobin concentration and indolent development of symptoms. However, this insidious onset may lead to physicians' underappreciation of the toll that fatigue, related to anemia, takes on their patients. [1]

The primary cause of anemia in children with cancer is decreased erythrocyte production. This may be due to replacement of normal hematopoiesis by malignant cells (leukemic blasts or metastatic solid tumor cells), transient marrow aplasia due to chemotherapy, or suppression of erythropoiesis due to inflammation, either related to the malignancy itself or accompanying infection (e.g., anemia of infection or chronic disease). Acute or chronic blood loss facilitated by concomitant thrombocytopenia also may occur. Rarely, hemolysis due to chemotherapeutic agents,[2] infection, or alloantibodies induced by prior transfusions may occur as well. [3]

If the bone marrow is not producing red blood cells, the rate of decline in the hemoglobin concentration should be 1/120th of the red cell mass per day, or approximately 0.7 to 1.0 g per dL per week. A more rapid decline in hemoglobin suggests bleeding or hemolysis. With marrow suppression, the reticulocyte count is inappropriately low for the degree of anemia, and leukopenia, thrombocytopenia, or both are common. Children with cancer also often have a component of the anemia of inflammation or chronic disease, characterized by defective iron recycling, relatively decreased serum erythropoietin concentrations, and inhibition of erythropoiesis by such cytokines as tumor necrosis factor (TNF) and interleukin-1b (IL-1b). [4] Thus, patients with newly diagnosed solid tumors, even without marrow infiltration, may have a mild hypoproliferative anemia.

Parvovirus B19, the cause of erythema infectiosum (also known as *fifth disease*) in healthy children and transient aplastic crises in patients with chronic hemolytic anemia, may produce prolonged erythroid aplasia in immunosuppressed individuals. The primary infection may be transmitted by respiratory droplet contact or transfusion.[5,6] Persistence of parvovirus may inhibit erythropoiesis for months.[7,8] Neutropenia and thrombocytopenia also may occur, interfering with continued chemotherapy.[5,9] Repeated infusion of intravenous immunoglobulin may provide neutralizing anti-B19 antibodies, resulting in more rapid return of erythropoiesis. [10] Persistence of infection due to other viruses, such as cytomegalovirus (CMV) or Epstein-Barr virus (EBV), also may lead to erythroblastopenia or pancytopenia. In such cases, specific antiviral therapy and immunoglobulin treatment may be beneficial in treating the infection and reestablishing hematopoiesis.

Bleeding in association with thrombocytopenia, most commonly from epistaxis or gastrointestinal hemorrhage, usually is clinically evident. However, the volume of blood loss from occult gastrointestinal bleeding or repetitive blood sampling for laboratory monitoring may be inapparent and substantial.

Hemolysis is an unusual mechanism of anemia in pediatric cancer patients ( Table 40-1). Sepsis and disseminated intravascular hemolysis (DIC) are clinically evident causes. Bone marrow transplant conditioning or prior intensive chemotherapy may produce a thrombotic microangiopathy resembling thrombotic thrombocytopenic purpura (TTP) or hemolytic uremic syndrome (HUS) that may be more cryptic in presentation. [2,11]

| Pathophysiology | Laboratory finding | Clinical example |
|---|---|---|
| Microangiopathy | Blood smear: schistocytes, helmet cells | DIC (sepsis, APML); thrombotic microangiopathy after BMT[456] or chemotherapy[2] |
| Immune mediated | Blood smear: microspherocytes, positive direct antiglobulin (Coombs') test | Hodgkin's disease; drugs (teniposide, mitomycin-C)[2,11]; transfused ischemagglutinins[1]; alloantibodies[39] |

APML, acute promyelocytic leukemia; BMT, bone marrow transplantation; DIC, disseminated intravascular coagulation.

**TABLE 40-1. HEMOLYTIC ANEMIA IN CHILDREN WITH CANCER**

Immune-mediated hemolysis may result from untreated Hodgkin's disease, thymoma, a chemotherapeutic agents such as teniposide, or prior transfusions. Delayed hemolytic transfusion reactions, due to pretransfusion antibody titers that have waned to undetectable levels, are increasingly problematic with aggressive treatment of multiple relapses. Mild immune-mediated hemolysis may result from isohemagglutinins in the plasma in which transfused platelets are suspended. [3] This last problem can be avoided by use of ABO and Rh type-specific platelets.

## Indications for Erythrocyte Transfusion Support

Red blood cells should be transfused to maintain oxygen-carrying capacity rather than empirically to maintain an arbitrary value. When a patient is otherwise healthy and not dehydrated, oxygen delivery usually is sufficient above a hemoglobin concentration of 7.0 g per dL, a number recently endorsed by a National Institutes of Health Consensus Panel on perioperative transfusion. [12,13] Others have demonstrated that healthy adults may be able to tolerate hemoglobin concentrations as low as 5.0 g per dL without inadequate tissue oxygen delivery. [14] Indeed, avoiding transfusion may even be beneficial for certain patients. A large Canadian study demonstrated that severely ill intensive care unit patients randomly assigned to receive transfusion only if their hemoglobin concentration declined to below 7.0 g per dL had mortality lower than that in those who received red cell transfusions for a hemoglobin value below 10 g per dL. [15] However, no objective criteria exist by which to determine when to transfuse red cells to individual patients or how to evaluate the effects of that therapy. [16,17]

The child's disease state, coexisting thrombocytopenia, planned invasive procedures, and status relative to starting or recovering from a cycle of chemotherapy should be considered in assessing when to transfuse erythrocytes. When the hemoglobin value is below 6 to 7 g per dL, most children with cancer display symptoms of anemia, such as malaise, lassitude, decreased activity, or irritability that resolve with transfusion. With higher hemoglobin values, similar symptoms may occur without convincing evidence that anemia is the cause. Unless affected children have significant symptoms of anemia, transfusion need not be given routinely if leukemic remission or recovery from chemotherapy-induced aplasia is imminent. Conversely, if an invasive procedure is planned, particularly if such patients are significantly thrombocytopenic, a hemoglobin of approximately 10 g per dL may minimize significant perioperative bleeding by providing a cushion and by exercising a beneficial effect on hemostasis. [17,18] Similarly, red cell transfusion support should be considered for affected children who have a modest degree of anemia (hemoglobin of 8 to 10 g per dL) and are receiving or have just received intensive myelosuppressive chemotherapy, in preparation for the inevitable decline in both platelets and red cells.

## Erythrocyte Replacement Therapy

### *Whole Blood*

Although whole blood was the principal erythrocyte-containing product used before the 1960s, individual component therapy of packed red blood cells, single-donor platelets, or fresh frozen plasma (FFP) now is preferred. Whole blood rarely is available without advance planning and offers little advantage over packed erythrocytes plus crystalloid or FFP, even for treatment of hemorrhage or for exchange transfusion. [19]

### *Packed Red Blood Cells*

Each unit of packed red blood cells is prepared by the centrifugation of one unit of single-donor whole blood. The product is collected from a donor in varying volumes of anticoagulant and additives that determine the final hemoglobin concentration. A citrate-phosphate-dextrose-adenine anticoagulant (CPDA-1) commonly is used. CPDA-1-preserved units contain approximately 250 mL, with a hematocrit of 65% to 80% and may be stored for up to 35 days. [19,20] Additive solutions containing adenine and saline (Adsol or AS) may be added to anticoagulated packed red blood cells. This allows for storage of red cells for up to 42 days and is preferred for blood bank inventory reasons. Units of AS-added cells have a final volume of approximately 350 mL with a hematocrit of 55% to 60%. [19]

The volume of a red cell transfusion in children should be ordered in mL per kg of recipient body weight. The standard transfusion volume is 10 mL per kg. This should raise a recipient's hemoglobin by 2.5 to 3.0 g per dL or hematocrit by 8% to 9% if CPDA-1-anticoagulated cells are used and should raise the hemoglobin by 2.0 g per dL or the hematocrit by 6% to 7% if AS-preserved cells are used. The maximum volume of erythrocytes that can be administered safely in a 4-hour transfusion generally is 15 mL per kg in hemodynamically stable patients. Neonatal intensive care units may employ up to 17 mL per kg as their standard volume. Each episode of transfusion must be completed within 4 hours after the unit or aliquot has been entered.

Children with a hemoglobin concentration of less than 5 g per dL, particularly if signs of congestive heart failure or hypertension are present, should receive smaller repeated transfusions, perhaps accompanied by a diuretic. [21] A safe initial transfusion volume is the number of mL per kg equal to the hemoglobin value given over 4 hours. That is, if the hemoglobin value of a 10-kg child is 4 g per dL, 40 mL of packed cells may be given over 4 hours without significant risk of volume overload. Multiple small-volume transfusions, separated by several hours to allow cardiovascular stabilization, can restore oxygen-carrying capacity within 24 hours. In small children, a single unit of red cells can be divided into sterile aliquots by the blood bank to provide several transfusions from one unit and, thus, one donor exposure. Rapid transfusion of a large volume of erythrocytes in such circumstances may result in pulmonary edema.

A partial exchange transfusion, either performed manually in aliquots of 10 to 50 mL or by automated erythrocytapheresis, may be preferable to repetitive transfusion for severe anemia. This procedure requires good vascular access but has the advantage of rapid isovolumetric correction of the anemia. The volume of blood for manual exchange can be estimated by a variety of formulas. [22,23]

### *Component Processing*

Leukocyte depletion and irradiation of red cells are reviewed later in this chapter.

### *Red Cell Substitutes*

Red cell substitutes, including human or bovine cell-free hemoglobin solutions, perfluorocarbon emulsions, or liposome-encapsulated hemoglobin, are being investigated in advanced clinical trials. Side effects are not understood completely but include both gastrointestinal complaints and vasoconstriction for hemoglobin derivatives and thrombocytopenia and dose limitations for the perfluorocarbon products. The major difficulty remains their very short (12- to 48-hour) duration of action, inadequate for most needs of pediatric oncology patients. [24]

### *Recombinant Human Erythropoietin Therapy*

The etiology of the anemia observed in children with cancer includes inappropriate erythropoietin production in response to a decreased hemoglobin level. [25] Cytotoxic chemotherapy contributes further to the impaired erythropoietin response to anemia. [26] Recombinant human erythropoietin has been shown to have efficacy in the treatment of anemia in adults and children with chronic end-stage renal disease [27,28] and [29] and has been evaluated for the treatment of the anemia of prematurity as well. [30,31] The use of erythropoietin in patients undergoing cytotoxic chemotherapy, therefore, is logical.

The specific uses of erythropoietin for pediatric patients undergoing chemotherapy remain poorly defined. Most studies of erythropoietin have focused on adult patients. In a series of double-blind placebo-controlled trials, thrice-weekly subcutaneous injections of erythropoietin, 100 to 300 units per kg per dose, generally resulted in a rise in hemoglobin concentration of 1 to 2 g per dL in patients receiving moderately intensive chemotherapy. [1,32,33,34,35,36,37,38,39,40,41,42] and [43] Small studies of pediatric patients undergoing moderate-dose chemotherapy suggested a similar effect. [36,43,44] Furthermore, studies of adults have suggested that the increase in hemoglobin concentration caused by erythropoietin may be associated with an improved quality of life. [32,42,45] Studies currently under way are assessing the effect of

erythropoietin on the quality of life of children who are undergoing chemotherapy for solid tumors or leukemia.

Randomized trials evaluating erythropoietin use in patients undergoing allogeneic bone marrow transplantation (BMT) have demonstrated that erythropoietin therapy can hasten erythroid engraftment.[46,47,48 and 49] However, the effect is modest at best, and the cost of the erythropoietin use exceeds the cost of the erythrocyte transfusions. In the setting of autologous BMT, randomized studies have not found any reduction in red blood cell transfusion requirements or time to erythrocyte engraftment with erythropoietin use.[50,51] Nonrandomized studies of pediatric patients undergoing either autologous or allogeneic BMT report that the efficacy of erythropoietin is similar to that reported in adult trials. [43,52]

Despite these data, the role of erythropoietin therapy in the supportive care of pediatric cancer patients remains undefined. The agent is expensive, requires an additional injection for patients who already may be receiving other cytokines, and may not be effective in patients whose chemotherapy treatments are intensive. The quality-of-life benefits seen in adult patients may not pertain to children. Given the increasing safety of red blood cell transfusions (see Complications of Blood Transfusion Support), the advantage of erythropoietin therapy with regard to enhanced safety might be considered marginal. However, in certain groups of patients, such as Jehovah's Witnesses, erythropoietin may play an important role.

## HEMOSTASIS

Normal hemostasis *in vivo* is maintained by a balance between hemorrhagic and thrombotic regulatory pathways. Pediatric cancer patients are at risk for impairment of hemostasis as a result of thrombocytopenia, platelet dysfunction, DIC, and liver disease and the prothrombotic tendencies of common therapies, such as L-asparaginase or indwelling central venous lines. Overall, thrombocytopenia is the most common complication leading to hemorrhage encountered by pediatric oncologists, and the primary form of hematologic support is platelet transfusion.

## THROMBOCYTOPENIA

### Pathophysiology

Thrombocytopenia, defined as a platelet count of less than 150,000 per $mm^3$, results from one or more of four basic mechanisms: decreased production, increased destruction, hypersplenism (i.e., splenic sequestration), or consumption that accompanies brisk bleeding or extensive transfusion. Decreased production of platelets usually is caused either by marrow replacement by leukemia or by solid tumor, or marrow suppression secondary to chemotherapy or infection.

Peripheral destruction of platelets with inadequate marrow compensation may occur on an immune basis or may be due to mechanical factors. Immune-mediated thrombocytopenia has been described in children with leukemia and other forms of cancer, but its occurrence may be coincidental. [53,54] Immune complexes may be deposited on the platelet membrane during infection and can result in thrombocytopenia from ingestion of the platelet by mononuclear phagocytes in the spleen. The thrombocytopenia that may occur after dactinomycin (actinomycin-D) therapy appears to be immunologic and responds to treatment with prednisone. [55]

Thrombocytopenia from mechanical platelet injury occurs in DIC, complicating acute promyelocytic leukemia (APML) or solid tumors, such as neuroblastoma. Significant thrombocytopenia from hypersplenism is uncommon except in the massive chronic splenomegaly seen in such disorders as Langerhans' Cell histiocytosis, hemophagocytic syndromes, and juvenile chronic myelogenous leukemia.

### Indications for Platelet Transfusion Support

There is no doubt about the need for platelet support in children who have cancer and have a platelet count below 50,000 per $mm^3$ and extensive cutaneous mucosal or internal bleeding.[56] Patients with cancer and platelet counts between 20,000 and 100,000 per $mm^3$ may be at risk of hemorrhage with invasive procedures, such as lumbar puncture or surgical incision. A minimum platelet count of 50,000 per $mm^3$ has been recommended widely for surgical procedures on the basis of consensus rather than scientific evidence.[57] Similarly, a platelet count of 20,000 per $mm^3$ often is advised for lumbar puncture. A minimum platelet concentration is not needed before bone marrow aspiration or biopsy, as direct pressure can control bleeding.

The more difficult questions are the degree of thrombocytopenia that should trigger prophylactic platelet transfusions and the considerations other than platelet number that should affect management decisions regarding transfusion use. Life-threatening hemorrhage, either intracranial or gastrointestinal, are fortunately too rare to serve as an end point for therapeutic trials of prophylactic platelet transfusion. Thus, designing prospective randomized clinical trials with clinically relevant end points is difficult. Consensus guidelines do exist, but they are conflicting and, in practice, may not always be followed. [56,58,59] In the United States today, approximately 75% of platelet concentrates are given for prophylaxis rather than as treatment of overt bleeding. [57,60]

In 1962, Gaydos et al.[61] established a quantitative relationship between platelet count and significant hemorrhage in patients with newly diagnosed acute leukemia. Although the frequency of days during which bleeding was observed increased slightly with a platelet count below 20,000 per $mm^3$, no threshold that predicted hemorrhage was found.[61,62] However, in the early 1960s, most patients would have been treated with aspirin routinely, rendering the data inapplicable to pediatric patients of today.[62] In 1978, Slichter and Harker[63] produced the first good evidence of a clinical platelet threshold below which bleeding increased. Using a sensitive radioisotopic technique, they demonstrated an increase in stool blood loss only with platelet counts below 5,000 to 7,000 $mm^3$.

Two descriptive studies subsequently examined the risk of thrombocytopenic bleeding and found an increase in hemorrhage only if the platelet count was below 10,000 per $mm^3$.[64,65] Four other studies more recently assessed the risk of bleeding when transfusions were given for measured platelet counts below 10,000 per $mm^3$ versus below 20,000 per $mm^3$. All studies demonstrated the absence of difference in bleeding episodes, number of patients with bleeding, or deaths caused by hemorrhage between the groups.[66,67,68,69,70 and 71] Administering platelet transfusions only for overt bleeding or for a platelet count below 10,000 per $mm^3$ resulted in a mean reduction of 30% (range, 22% to 40%) in the total number of platelet transfusions across all studies. [71]

Similar findings emerged from studies of patients undergoing BMT. Specifically, no difference was seen in bleeding events regardless of assignment to prophylactic platelet transfusion below either 10,000 per $mm^3$ or 20,000 per $mm^3$. Further, hemorrhagic cystitis, mucositis, infection, and graft-versus-host disease—all complications of transplantation that increase the bleeding tendency—were present in nearly all patients who experienced major hemorrhages. [66,72] This finding suggests that clinical status rather than platelet count can predict who will have bleeding complications, even in high-risk BMT patients. Thus, more intensive platelet transfusion support could be reserved for patients with these other risk factors for bleeding.

On the basis of these studies, a new consensus has been formulated, as summarized in Table 40-2. For stable cancer patients, a platelet threshold of 5,000 to 10,000 per $mm^3$ is considered safe, and prophylactic platelets should be given only if the platelet count falls below this level. [59,60,62,64,71,73,74] Assuming that patients have normally functioning platelets, fatal bleeding is unlikely to occur at a platelet count over 5,000 per $mm^3$.[59,65] However, if children have other risk factors for thrombocytopenic bleeding, including infection, coagulopathy, a sudden increase in cutaneous hemorrhage, oral blood blisters, retinal hemorrhages, or a rapidly falling platelet count, transfusion to maintain a platelet count of 20,000 per $mm^3$ is suggested.[59,60,62,64,71,73,74]

| Clinical status | Platelet count per $mm^3$ | Intervention |
|---|---|---|
| Well | >5,000–7,000 | Observe for bleeding |
| Febrile, stable | <10,000 | Transfusion |
| Mucosal bleeding or febrile, unstable | <20,000 | Transfusion |
| Extensive mucosal or internal bleeding | <50,000 | Transfusion |
| Invasive procedure | | |
| Surgery | <50,000 | Transfusion |
| Lumbar puncture | <20,000 | Transfusion |
| Bone marrow | <5,000 | Transfusion |

**TABLE 40-2. PLATELET TRANSFUSION TRIGGERS IN CHILDREN WITH CANCER**

## Platelet Transfusion

### *Random-Donor Platelet Concentrates*

A unit of platelets is separated from a single-donor unit of whole blood by centrifugation. The platelets then may be stored for up to 5 days at 24°C with continuous agitation to prevent clumping. Each unit contains approximately 5.5 to 10.0 × $10^{10}$ platelets in 40 to 70 mL of plasma and anticoagulant preservative.[19,20] When more than one unit of random-donor platelets is ordered, the blood bank generally pools the individual units for ease of administration. However, in very young children or when volume overload is a concern, the blood bank can further concentrate the pooled platelets by gentle centrifugation on request. This results in a 10% to 20% loss of platelets in the transfused product.[20]

The volume of platelets to be transfused depends on the weight of the child and the post-transfusion platelet count desired. In previously nontransfused patients, 1 unit per $m^2$ of body surface area should raise the 1-hour post-transfusion platelet count by 10,000 to 12,000 per $mm^3$. Thus, 1 unit per 6 to 8 kg of body weight should increase the platelet count by 40,000 to 60,000 per $mm^3$ over the pretransfusion value. For practical purposes, a minimum of 2 units of platelets should be used for infants, 4 units for young children, and 6 units for adolescents.

Achieving the normal 7- to 10-day survival for transfused platelets rarely is attainable. Prior blood product administration may have led to formation of alloantibodies to human leukocyte antigen (HLA) class 1 or platelet-specific antigens, thus promoting immune destruction of the transfused platelets.[75] Alternatively, patients may consume the transfused platelets to stop hemorrhage or in processes such as DIC. To determine why a posttransfusion platelet increment is less than expected, platelet values 1 hour and 8 to 24 hours after the transfusion can be compared. If patients are alloimmunized, both the 1-hour and 8- to 24-hour posttransfusion values will be inappropriately low and somewhat similar. If the platelets are being consumed, the 1-hour value will be notably higher than the 8- to 24-hour measurement.

Fortunately, the incidence of platelet refractoriness appears low, even in the intensively transfused BMT recipient.[76,77 and 78] Administration of single-donor apheresis platelets (see Apheresis Platelets) and leukoreduction to fewer than 5 × $10^6$ residual leukocytes per unit, particularly if performed before storage, may reduce further the incidence of alloimmunization.[79] ABO and Rh type-specific platelets will survive longer in the recipient and should be used whenever possible.[80] Platelet cross-matching by a variety of techniques may allow also for selection of platelet products likely to result in improved posttransfusion counts.[81] Intravenous immunoglobulin and protein A column immunoabsorption therapy are of limited effectiveness in raising platelet transfusion increments in patients with platelet alloantibodies.[82,83]

### *Apheresis Platelets*

Increasingly, single-donor platelets are being obtained by mechanical apheresis. The donors, who must have adequate veins, spend approximately 90 minutes having their platelets removed by differential centrifugation, and red cells are returned. Apheresis platelets may be donated every 5 to 15 days as compared to the 8-week minimum interval for whole blood donation. Although dependent on the instrument and software used for collection, apheresis platelet units contain approximately 3 to 6 × $10^{11}$ platelets. This is equivalent to 6 to 10 units of random-donor platelets in a volume of 200 to 300 mL.[84] Apheresis products now account for more than 50% of all platelet concentrates transfused in the United States.[60] For smaller patients, apheresis units may be divided using sterile technique in the blood bank, with one-fourth or one-half of an apheresis unit then being transfused instead of 2 to 4 random-donor units. In cases of platelet refractoriness, HLA-matched or platelet cross-match-compatible donors can be collected by apheresis for specific recipients.[84]

If available, platelets obtained by apheresis are preferred to random-donor units, as they reduce the number of donors to whom the patient is exposed, thereby decreasing the risk of alloimmunization and transfusion-transmitted diseases.[60] However, the Trial to Reduce Alloimmunization to Platelets (TRAP), which compared methods of leukocyte reduction (single-donor filtered vs. unfiltered vs. filtered apheresis) from platelets, could not document reduced alloimmunization with apheresis platelets as compared to pooled single-donor concentrates if both were filtered.[85]

### *Methods of Transfusion*

Platelets can be infused rapidly by gravity over 20 minutes when the volume status allows. Apheresis units may be given over 20 to 240 minutes as clinically indicated. As with all blood products, transfusion must be completed within 4 hours of entry into the unit. For patients who are refractory to platelets and have ongoing bleeding, a constant infusion of platelets may be beneficial. In this approach, either one or two random-donor units or one-half of an apheresis unit of platelets is administered slowly over 4 hours. Administering platelets in this manner may not increase the platelet count but should enhance hemostatic efficacy.

## Agents with Thrombopoietic Activity

Agents that stimulate patients' own production of platelets are a potentially promising new approach to minimize the need for allogeneic platelet transfusion in children with cancer. Colony stimulating factors (CSFs) such as granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) do not have thrombopoietic activity in clinical trials.[86,87,88,89,90,91,92,93 and 94] A variety of other agents have been studied, including IL-1,[95,96,97 and 98] IL-3,IL-6,[97,98,99,100,101,102,103 and 104] IL-11, and thrombopoietin. Clinical trials evaluating these agents demonstrate only a modest decrease in platelet transfusion requirements.[105] Accordingly, their role in the treatment of patients undergoing myelosuppressive chemotherapy or stem-cell transplantation is unknown. Phase III trials for some of these agents are in progress. Hematopoietic growth factors currently licensed in the United States, and the indications for which they were approved, are summarized in Table 40-3. The following sections review those agents most recently under clinical investigation.

| | | | |
|---|---|---|---|
| G-CSF | Chemotherapy-induced neutropenia; neutropenia after stem-cell transplantation; chronic or congenital neutropenia; peripheral blood stem-cell mobilization | Pediatric and adult | 5–10 μg/kg per day i.v. or s.c. |
| GM-CSF | Chemotherapy-induced neutropenia; neutropenia after stem-cell transplantation; peripheral blood stem-cell mobilization | Pediatric and adult | 250–500 μg/$m^2$ per day i.v. or s.c. |
| Erythropoietin | Chemotherapy-induced anemia; anemia due to chronic renal failure; anemia due to zidovudine therapy in HIV patients; reduction of blood transfusions in surgical patients | Pediatric and adult | 50–300 units/kg i.v. or s.c. 3 times weekly |
| Interleukin-11 | Prevention of chemotherapy-induced thrombocytopenia and reduction in the need for platelet transfusions in nonmyeloid malignancies | Pediatric and adult | 25–100 μg/kg per day s.c.<br>50 μg/kg per day s.c. |

G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HIV, human immunodeficiency virus.

**TABLE 40-3. HEMATOPOIETIC GROWTH FACTORS CURRENTLY LICENSED BY THE U.S. FOOD AND DRUG ADMINISTRATION**

## Interleukin-3

IL-3 belongs to a family of glycoprotein hormones that have been shown to modulate proliferation and functional activity of hematopoietic cells *in vitro* and *in vivo*.[106] It is produced by activated T cells, natural killer cells, and mast cells.[107,108 and 109] In preclinical models, IL-3 was shown to stimulate myelopoiesis, erythropoiesis, and thrombopoiesis.[107,110,111] When given in combination with GM-CSF, IL-3 has been shown *in vitro* to have a synergistic effect on progenitor cell cycling rates and results

in an increase in white blood cell counts as compared to GM-CSF alone. [112]

Phase I trials of IL-3 both as a single agent or combined with other hematopoietic cytokines have been performed in patients with advanced malignancy,[113,114,115,116,117,118,119,120,121,122 and 123] secondary hematopoietic failure,[124] and myelodysplastic syndromes.[124,125] IL-3 has also been studied in children with nonmalignant disorders, such as aplastic anemia,[124,125,126,127 and 128] Diamond-Blackfan Anemia,[129,130 and 131] Fanconi's Anemia,[130] and amegakaryocytic thrombocytopenia.

Clinical trials have shown only a modest decrease in platelet transfusion requirements with IL-3 use in patients receiving myelosuppressive chemotherapy. The most favorable of these studies suggested that IL-3 may shorten the time to both platelet and neutrophil recovery, but other reports describe little or no response. [105] One study showed no cost savings associated with use of IL-3 in patients undergoing autologous BMT. [132] Several studies have shown that sequential IL-3 and GM-CSF may be used safely in the collection of peripheral stem cells, although phase III trials to assess the contribution of the IL-3 are not available. [133,134] IL-3 also has been combined sequentially with GM-CSF in patients receiving chemotherapy [135,136,137 and 138] or undergoing autologous BMT.[139,140] These studies have shown variable results when the contribution of IL-3 to platelet recovery is evaluated. Adverse effects of IL-3 include fever, headache, chills, bone pain, facial swelling, and mild local erythema at the site of subcutaneous injection.[113,115]

**PIXY-321**

PIXY-321 is a fusion protein consisting of human recombinant GM-CSF and human recombinant IL-3 coupled by a flexible amino acid linker sequence that allows the binding domains to fold into their native conformation.[141] PIXY-321 binds to cells via either its GM-CSF or IL-3 domains and has comparable or greater affinity for GM-CSF or IL-3 receptors in competitive binding studies than does either protein alone. [142] It stimulates the proliferation of normal human megakaryocyte and myeloid progenitors[143] and induces bilineage platelet and neutrophil production in preclinical models. [144]

PIXY-321 has been studied in several phase I studies in humans, and minimal toxicity has been encountered. [145,146,147,148 and 149] After chemotherapy, PIXY-321 reduces the incidence of severe neutropenia but, after several cycles of therapy, more severe thrombocytopenia occurs than is seen in untreated controls. [150] Further, 92% of patients developed neutralizing antibodies after two courses of PIXY-321. [151] Interest has waned in this use of the agent, and it no longer is being produced.

**Interleukin-11**

IL-11 exerts its primary effect on maturation of megakaryocytes by increasing their ploidy. [152,153] It exerts a synergistic effect when combined with other early-acting hematopoietic growth factors, such as IL-3 and stem-cell factor.[153,154,155 and 156] Inpreclinical trials, IL-11 stimulated platelet production in mice, [153,157] and the response was characterized by an increase in platelet counts, peaking at approximately 14 to 21 days after initiation of therapy. Multilineage effects also were seen in these models.[157]

IL-11 has been studied both in patients undergoing chemotherapy and in the autologous bone marrow setting. Women undergoing chemotherapy for breast cancer were treated with doses ranging from 10 to 100 µg per kg per day for 14 days during a 28-day prechemotherapy safety trial. IL-11 was associated with mean 76%, 93%, 108%, and 185% increases in platelet count at doses of 10, 25, 50 and 75 µg per kg per day, respectively. [158] However, a randomized trial in patients with breast cancer undergoing autologous BMT did not demonstrate a decrease in platelet transfusions in women who received 25 or 50 µg per kg per day of IL-11 as compared with placebo.[159] IL-11, at doses of 25, 50, and 75 µg per kg combined with G-CSF, was administered to children who received ifosfamide, carboplatin, and etoposide. In this phase I/II study, apparently the use of IL-11 decreased the number of platelet transfusions and accelerated platelet recovery as compared with historical controls.[160]

Adverse reactions observed among patients receiving IL-11 have included edema, transient anemia, arrhythmias, fever, chills, fatigue, myalgias, and headache. Manifestations of heart failure (dyspnea, edema, conjunctival injection, increased pleural effusions) were observed and likely were due to the increased intravascular volume that results from renal sodium retention, hypotension, and allergic reactions with IL-11 administration. [161] IL-11 currentlyis approved by the U.S. Food and Drug Administration (FDA) for the prevention of severe chemotherapy-induced thrombocytopenia after myelosuppressive chemotherapy in patients with nonmyeloid malignancies. It is not indicated for patients receiving myeloablative chemotherapy prior to stem-cell transplantation. The recommended dose is 50 µg per kg per day in adults and 75 to 100 µg per kg per day in children.

**Thrombopoietin**

Thrombopoietin is a hematopoietic growth factor that regulates megakaryopoiesis through its interaction with a specific cell-surface receptor encoded by the c- *mpl* proto-oncogene.[162,163,164,165 and 166] Thrombopoietin also acts on the erythroid lineage and primitive hematopoietic progenitors as a synergistic growth factor. [167,168] In mice, injection of exogenous thrombopoietin increased platelet counts fourfold to sixfold within 1 week but had no effect on leukocyte or red cell numbers. [168,169 and 170] In nonhuman primates, thrombopoietin resulted in increased platelet counts, with a peak between 12 and 14 days after initiation of treatment. [171]

Two molecules currently are undergoing clinical trial: a recombinant human thrombopoietin identical to the native molecule (rHuTPO) developed by Genetech (San Francisco, CA) and a recombinant nonglycosylated molecule (MGDF) developed by Amgen (Thousand Oaks, CA). MGDF contains the receptor-binding portion of the native molecule coupled to polyethylene glycol to protect it from degradation. [105] Adverse events encountered with the use of MGDF and rHuTPO included nausea, arthralgia, deep venous thrombosis, and pulmonary embolism. Injection-site reactions occur but appear to be rare. [105]

Clinical experience with these agents has been limited. In a dose escalation study that randomly assigned patients to MGDF or placebo after chemotherapy, a dose-dependent, lineage-restricted increase in platelet counts was seen. Recovery to baseline platelet count was seen in 17 days for the MGDF-treated patients versus 22 days for placebo.[172] In a second study, 53 patients with lung cancer were treated with MGDF in a dose-escalating fashion. Patients who received MGDF had higher median nadir platelet counts and platelet count recovery to baseline in 14 days versus 21 days in those receiving placebo. [173] In a phase I trial of rHuTPO, a single dose of 2.4 mg per kg was administered to 12 patients with sarcoma, and platelet counts rose 3.6-fold at 12 days. Platelet function was normal, and no adverse reactions were reported.[165] Randomized trials are under way to determine the efficacy of these agents in decreasing the duration of thrombocytopenia and the need for platelet transfusions.

The impact of thrombopoietic growth factors on clinical practice has been limited, and their efficacy has been modest at best. Whether newer agents will be shown to have an impact on the degree of thrombocytopenia and the use of platelet transfusion remains to be seen.

***Other Treatments for Thrombocytopenia***

In addition to platelet transfusion support, other nonspecific measures may be of value for patients with severe thrombocytopenia. Avoidance of invasive procedures, such as insertion of urinary catheters and nasogastric tubes, intramuscular injections, rectal examinations, and deep venipunctures as well as application of firm local pressure for 5 to 15 minutes after epistaxis or puncture wounds will minimize bleeding complications. Further, antiplatelet agents, such as ibuprofen, ketoprofen, or aspirin should be avoided by patients with severe thrombocytopenia, particularly if bleeding is present.

P>Epsilon-aminocaproic acid (Amicar), a fibrinolytic inhibitor, would not be expected to enhance primary hemostasis greatly, but some clinicians attest to the utility of antifibrinolytic agents in thrombocytopenic patients with mucosal hemorrhage. [174] Prophylactic use of the antifibrinolytic agent tranexamic acid in one study has been shown in one study to decrease the use of platelet transfusions in (AML) patients receiving chemotherapy. [175] Topical agents such as Gelfoam or topical thrombin also may promote local hemostasis in thrombocytopenic patients. Desmopressin enhances the adhesion of platelets to vascular endothelium and shortens the bleeding time in mildly thrombocytopenic patients. It is thought to be of questionable value in patients with platelet counts below 50,000 per mm $^3$.[176,177] However, one study suggested that desmopressin controlled hemorrhage even in markedly thrombocytopenic children. [178]

Prednisone in low doses may reduce hemorrhage in thrombocytopenic patients as a consequence of its nonspecific effects on capillary stability. [179] Estrogen or

high-dose birth control pills may be of value for menometrorrhagia.

## COMPLICATIONS OF BLOOD TRANSFUSION SUPPORT

### Immunologic Complications

Every blood product transfusion carries a risk of an untoward event. Immunologic and infectious events are the most common. The current estimate of risk for each type of common transfusion-related event is summarized in Table 40-4.

| Infection or event type | Rate | Reference |
|---|---|---|
| Human immunodeficiency virus | 1:660,000* | Goodnough,[19] Schreiber[181] |
| Hepatitis A | 1:1,000,000 | Schreiber[215] |
| Hepatitis B | 1:63,000 | Schreiber[215] |
| Hepatitis C | 1:125,000* | Holland[213] |
| Human T-cell leukemia virus I and II | 1:641,000 | Goodnough,[19] Schreiber[181] |
| Bacterial contamination: | | |
| Red blood cells | 1:65,000–500,000 | Goodnough,[19] Blajchman[198] |
| Platelets | 1:12,000 | Goodnough[19] |
| Transfusion reactions: | | |
| Acute hemolytic transfusion reaction (fatal) | 1:250,000–1,000,000 | Linden[196] |
| Delayed hemolytic transfusion reaction | 1:1,000 | Noy[93] |
| Transfusion-related acute lung injury | 1:5,000 | Goodnough[19] |

*May be decreased further by nucleic acid testing (see text).

**TABLE 40-4. ESTIMATED RISK OF EVENT PER UNIT OF BLOOD PRODUCT TRANSFUSED**

### *Transfusion Reactions*

Fatal hemolytic transfusion reactions continue to occur at a rate of 1 in 250,000 to 1,000,000 transfusions. [180] Approximately one-half are due to acute hemolytic reactions from ABO incompatible transfusion caused by administrative errors (e.g., mislabeled specimens or administering blood to the wrong patient). [19,181] Less severe acute hemolytic transfusion reactions also are encountered. These reactions manifest as fever, chills, back or abdominal pain, dark urine, pallor, bleeding, or shock during the transfusion. Spherocytes are present on the peripheral blood smear; plasma free hemoglobin and bilirubin will rise; the urine will show hemoglobinuria (dipstick positive for blood but no red cells on microscopy); and a positive direct antiglobulin test will occur. [182] Clinical features of common immune transfusion reactions are reviewed in Table 40-5. In the presence of suspected reaction, the transfusion should be stopped, and the remaining product, plus a post-transfusion sample of the patient's blood, should be sent to the blood bank for analysis. Management consists of vigorous hydration to maintain urine flow until hemoglobinemia and hemoglobinuria resolve. [19]

| Type of Reaction | Incidence (%) | Antibody Directed Against | Symptoms |
|---|---|---|---|
| Acute hemolytic | <0.01 | ABO antigens | Fever, hemolysis |
| Non-hemolytic, febrile | 0.5–1.0 | HLA antigens on white cells | Fever, headache, chills |
| Allergic | 0.25 | Plasma proteins | Hives |
| Anaphylactic | 1 in 700 | IgA | Stridor, periorbital swelling |

IgA, immunoglobulin A.

**TABLE 40-5. TYPES OF TRANSFUSION REACTIONS**

Approximately 1 in 1,000 adult transfusion recipients have a delayed hemolytic transfusion reaction. These are characterized by low-grade fever, jaundice, and a hemoglobin increment lower than expected 2 to 14 days after transfusion. [19] Delayed hemolytic transfusionreactions occur in previously alloimmunized patients in whom transfused cells provoke the amnestic production of antibody that was not detectable by routine cross-match prior to transfusion. [19,183] Preventing these reactions is difficult, but good communication between physician and blood bank concerning a patient's history of prior transfusion outside the institution and review of transfusion records may minimize the risk.

Non-hemolytic transfusion reactions are the most frequent acute adverse event related to transfusions. Fever does not always occur during such episodes. These reactions can include components that are both inflammatory (chills, rigors, and discomfort, which may be accompanied by fever) and allergic (pruritus, urticaria, erythema, and flushing).[79] Classically, febrile nonhemolytic transfusion reactions were thought to be caused by antileukocyte antibodies in patients' plasma, resulting in release of endogenous pyrogens. Febrile nonhemolytic transfusion reactions to red cells still are believed to be caused by this mechanism, and removal of leukocytes by filtration or apheresis can decrease their frequency. [184]

Cytokines, such as IL-1, IL-6, and TNF-asecreted by the residual leukocytes in platelet concentrates, now are believed to be a major cause of platelet reactions. Commercially available filters cannot remove these inflammatory cytokines once they are formed in stored blood products. Thus, prestorage leukofiltration offers the most effective approach to preventing febrile nonhemolytic transfusion reactions due to platelet components. [184,185]

Febrile non-hemolytic transfusion reactions can begin at any time during or after completion of a transfusion. Therapy consists of stopping the transfusion temporarily and treatment with diphenhydramine and acetaminophen. If an allergic reaction is unusually severe—a so-called anaphylactoid reaction characterized by wheezing, bronchospasm, laryngeal edema, or hypotension—epinephrine and parenteral steroids should be considered, and the other therapies should be repeated. [19,186] If a reaction continues or recurs when the transfusion is restarted, the remainder of the product should be discarded. Patients who repetitively experience febrile nonhemolytic transfusion reactions will benefit from blood product leukofiltration (discussed in the section Leukocyte Depletion) and premedication with the same agents.[19] The most severe such reactions occur in the 1 in 700 individuals who are IGA–deficient and have been given immunoglobulin A–positive products. [187]

### *Transfusion-Related Acute Lung Injury*

Transfusion-related acute lung injury (TRALI) is a clinical syndrome usually occurring within 4 to 6 hours of transfusion of blood components containing plasma. It is characterized by dyspnea, hypoxia, fever, and noncardiogenic pulmonary edema. [186] It usually occurs in already sick patients and resembles adult respiratory distress syndrome. Thus, the recognized frequency of 1 in 5,000 transfusions is likely to be an underestimate. [181] It is most often associated with red cell and FFP transfusion from multiparous female donors. Transfused leukoagglutinins formed through prior exposure to paternal antigens during pregnancy are thought to interact with recipient white blood cells. These white cell complexes are sequestered in the pulmonary microvasculature, leading to increased vascular permeability and exudation of fluid and protein into the alveoli. [188] Biologically active lipids arising from donor blood cell membranes during storage also have been implicated as a contributory cause.[189]

Therapy for transfusion-related acute lung injury is supportive, with fluids to maintain blood pressure and cardiac output and supplemental oxygen or ventilatory support if needed. Pressor agents may be useful to manage hypotension. Corticosteroids are of marginal value. [186] Diuretics appear to be detrimental.[188] Mortality is

estimated to be between 5% and 10% of recognized cases.[188]

### *Special Considerations in Transfusion Therapy of Children with Cancer*

Unmodified erythrocyte and platelet products contain a large number of leukocytes that can induce alloimmunization. These HLA antibodies are responsible for most platelet refractoriness and may increase the chances of allograft rejection should a hematopoietic stem-cell transplant be needed. Furthermore, transfused immunocompetent donor lymphocytes may proliferate in immunosuppressed hosts, transmitting active CMV infection or causing graft-versus-host disease (GVHD). GVHD is discussed further in the section titled Transfusion-Associated Graft-Versus-Host Disease. The two approaches used most commonly to prevent these problems have been leukocyte depletion by filtration and irradiation of blood products before transfusion. [190]

### *Alloimmunization*

#### Leukocyte Depletion

Leukocytes can be removed from blood products by centrifugation, washing, freezing then thawing, and leukofiltration. [185] The first three techniques are labor intensive, involve obligate loss of product, and leave behind relatively large numbers (10% or more) of initial leukocytes. Commercially available red cell or platelet filters, although expensive, are simpler to use. Filtration removes 3 to 4 logs, or more than 99.5%, of white blood cells. [185,191]

Critical appraisal of numerous studies demonstrates that filtration can leukodeplete cellular blood products to below $5 \times 10^6$ leukocytes per unit, the level that is believed to minimize HLA alloimmunization.[79] This has reduced the frequency of HLA alloimmunization from between 70% to 97% in patients given nonfiltered products to 5% to 25% with filtration.[70,79,190] Variation in the frequency of alloimmunization may be related to whether filtration is employed before storage or at the bedside. Pre-storage filtration prevents the accumulation in the plasma of cytokines (TNF-a, IL-1b, IL-6, IL-8), leukocyte metabolites, and fragments that contribute to febrile nonhemolytic transfusion reactions.[192] Similarly, pre-storage—but not post-storage—leukofiltration has been shown to block the immunomodulatory solid tumor growth–promoting effect of transfusion reported in animal models.[185]

Reduction of leukocytes by filtration also decreases the transmission of CMV and, in theory, Creutzfeldt-Jakob prion disease in blood products from infected donors.[191,193] Leukofiltration, predominantly performed after storage at the bedside, has decreased the rate of acquisition of CMV in initially negative BMT and heavily treated leukemia patients to 0.5%. This is essentially the same as the 0.8% rate seen in recipients of unfiltered, serologically CMV-negative donor products and far below the 18% value for recipients of unscreened, unfiltered products. [191]

Leukodepletion conveys significant benefits to pediatric cancer patients, with data favoring pre-storage over bedside filtration. The United Kingdom has adopted universal prestorage filtration for all blood components, and similar proposals are being considered in the United States. Although filtration, which decreases alloimmunization and CMV transmission, is more cost efficient than CMV serotyping of donors (discussed in the section Cytomegalovirus), convincing cost-benefit analyses of the entire approach are not available.

#### Irradiation

Gamma irradiation of blood products with between 15 and 50 Gy will prevent leukocyte proliferation in a recipient without significant adverse effects on the transfused cells.[185] FDA regulations currently require 25 Gy to the center plane and 15 Gy to the entire container. [185] Platelet function appears undisturbed by this process. However, prolonged storage of red cells after irradiation may lead to increases in plasma potassium, adenosine triphosphate, pH, lactate dehydrogenase, and plasma free hemoglobin. Although some investigators recommend that gamma-irradiated red cells be washed before intrauterine transfusion, this does not appear to be necessary in other settings.[185,194]

The Trial to Reduce Alloimmunization to Platelets (TRAP) compared the effect of leukofiltration to ultraviolet B (UV-B) irradiation of platelet concentrates in nonalloimmunized AML patients.[85] Investigators found no difference in the rate of HLA alloimmunization between patients whose transfused platelets were filtered (18%) and those whose platelets were treated with UV-B irradiation (21%). By comparison, patients who received nonfiltered, nonirradiated platelets had a much higher rate of alloimmunization (45%). Similarly, the rate of acquired platelet refractoriness was equivalent in the filtered (8%) and the irradiated (10%) groups, and the rates in both were lower than in the control group (16%). The study concluded that filtration and UV-B irradiation of platelets are equally effective in preventing HLA alloimmunization.[58,85,190]

#### Rh Incompatibility

Whenever possible, Rh-matched red cell and platelet products should be used. [19] Although Rh antigens are not expressed on platelets, Rh-positive platelets (or red cells) should not be given to Rh-negative female recipients who are likely to survive into adulthood. If this becomes necessary owing to lack of Rh-negative product, administration of an anti–D immunoglobulin product immediately thereafter has been advocated. However, no studies documenting the utility of this strategy have been performed. Rh sensitization of immunosuppressed cancer patients is infrequent (7.8% in an early series). [195]

#### Transfusion-Associated Graft-Versus-Host Disease

Transfusion-Associated GVHD occurs when immunocompetent passenger T lymphocytes in cellular blood products engraft in a recipient. Clinical manifestations begin 4 to 30 days after transfusion and include fever and an erythematous maculopapular skin rash. Anorexia, nausea, vomiting, watery or bloody diarrhea, and elevated liver enzymes and bilirubin may be seen.[196,197] Eventually, this process results in severe bone marrow aplasia. It is associated with serious morbidity and mortality in 75% to 90% of affected patients.[197,198]

Originally described in children with immunodeficiency and in newborn recipients of *in utero* transfusion, numerous cases of GVHD in heavily immunosuppressed bone marrow transplant recipients have been reported.[197] In rare cases, it has been recognized in patients receiving chemotherapy for neuroblastoma, Hodgkin's and non-Hodgkin's lymphoma, acute lymphoblastic and myelocytic leukemia, and chronic lymphocytic leukemia. [197,199] Recognition of cases in immunocompetent individuals who share an HLA haplotype with HLA homozygous blood donors (i.e., relatives who provide directed donations or members of inbred populations) provides information regarding pathogenesis.[198]

Transfusion-associated GVHD may be prevented by gamma irradiation of blood products with 25 Gy. [198] Leukofiltration probably is not adequate prevention but has not been studied rigorously.[78] Irradiation of blood products to prevent transfusion-associated GVHD clearly is indicated for patients with immunodeficiency or recipients of *in utero* intrauterine transfusion, when the donor is a family member of the recipient, or if both are part of an inbred population. [197] Recipients of hematopoietic stem-cell grafts also should receive irradiated blood products. Available data do not support routine irradiation of all blood products given to pediatric cancer patients or to patients in whom a BMT is intended until the immunosuppression of the transplant conditioning begins. However, as a matter of convenience, leukofiltered and irradiated products often are prescribed routinely for all pediatric cancer patients, even for those who are not treated intensively or who may never need a bone marrow transplant. The cost of such products is the only disadvantage.

#### Infectious Complications

The infectious complications of blood transfusions in children with cancer are similar to those in children who do not have malignant disease and are exposed to a similar number of blood donors.

### *Bacterial Infection*

An underappreciated risk of transfusion is bacterial contamination during collection of transfusion products with a variety of microorganisms that proliferate during storage. These infections can cause fever and chills beginning during or shortly after a transfusion. Infection most commonly is due to *Yersinia enterocolitica* and other gram-negative organisms. Contamination usually is related to the length of storage, but *Yersinia* has been reported in red cells stored for only 1 week. Recent

incidence figures suggest that between 1 in 65,000 and 1 in 500,000 units transfused have bacterial contamination, with a 50% mortality in reported cases. [181,200]

Platelet units are contaminated most commonly with *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Staphylococcus epidermidis*. Approximately 1 in 12,000 platelet units are contaminated, far more often than red cell units, because bacteria proliferate readily during even 5 days' storage at 24°C. Overall mortality is 26% when reactions are reported due to bacterial contamination of platelets. Many cases may be missed because affected patients may have only mild symptoms and a blood culture positive with a common bacterial skin contaminant.[181] Management consists of prompt evaluation of any fever that begins within 6 hours after receipt of a blood product and administration of antibiotic therapy to patients who appear toxic. Preventative approaches using psoralens and ultraviolet-A light to produce nonimmunogenic and sterile blood products are being investigated. [181]

### *Parasitic Infection*

Although rare in the Western world, malaria, Chagas' disease (due to infection with *Trypanosoma cruzi*), and *Babesia* can be transmitted by transfusion. Global population movements and increasingly wide geographic distribution of pathogens have increased seroprevalence among unexpected donor populations, requiring awareness of the potential for transfusion transmitted infection with these unusual pathogens. [201,202]

### *Viral Infection*

With current pretransfusion testing, transmission of viral infections is extremely rare and thought to occur primarily during a window period: the interval soon after infection when a donor is infectious but when standard screening tests will be negative. [203] The estimated risks of infection now are lower than ever before and are expected to decrease further when PCR basednucleic acid testing for viral genomes (NAT testing) is fully implemented. [181,202,203 and 204] Each group of viruses is considered separately here.

#### Human Immunodeficiency Virus

Recognition that human immunodeficiency virus (HIV) infection could be transmitted by blood transfusion occurred in 1982. Even before implementation of HIV antibody testing in March 1985, high-risk donor voluntary deferral programs decreased the risk of infection per unit by nearly 3 logs. [181] Serologic testing for HIV-1 and HIV-2 decreased the per-unit risk to 1 in 493,000 by 1993.[205] In late 1995, p24 antigen testing further diminished the estimated risk of HIV infection per unit transfused to 1 in 676,000.[181] Nucleic acid testing should shorten the window period from 16 to 10 days and thus further decrease the risk of infection to approximately 1 in 1,000,000 units.[205] Nevertheless, transmission of HIV remains one of the most feared consequences of transfusion therapy.

#### Human T-Cell Lymphotrophic Virus I and II

Human T-lymphotrophic viruses (HTLV-1 and -2) are human retroviruses isolated from individuals with lymphoproliferative disorders, acute T-cell leukemia, and lymphoma. HTLV-1 has been associated also with myelopathy. HTLV-1 and HTLV-2 are proviruses and incorporate into host DNA, causing a lifelong carrier state. [206]

Transmission of HTLV-1 and HTLV-2 has been documented with cellular blood component transfusion. [181,206] Interestingly, donor lymphocytes that are required for transmission become noninfectious when stored, and they lose the ability to proliferate. [202] Thus, for this pathogen, blood product storage decreases the risk of infection. Specific screening antibody tests demonstrate a very low seroprevalence rate. [181,205]

Transfusion of HTLV-1 has resulted in myelopathy–tropical spastic paraparesis in 4% to 8% of recipients, although serologic evidence of infection will develop in 20% to 60% of recipients. One case of T-cell leukemia has been reported after HTLV-1–positive transfusion in a patient with acute myelogenous leukemia in remission.[181,182,183,184,185,186,187,188,189,190,191,192,193,194,195,196,197,198,199,200,201,202,203,204,205 and 206]

#### Hepatitis Virus and Posttransfusion Hepatitis

Hepatitis continues to result from blood transfusion in children with cancer. The specific viruses responsible for transfusion-related disease have progressed alphabetically from hepatitis B (HBV) to C (HCV) to G virus (HGV) and beyond. Since the 1943 report first describing posttransfusion hepatitis, a pattern of successive antigen identification and application of specific serologic testing of the blood supply has decreased the incidence of posttransfusion viral hepatitis due to all viruses from 23% of recipients in the 1960s to fewer than 1 in 10,000 recipients today. [207]

Transfusion-transmitted HBV infection declined markedly after introduction of all volunteer blood donors and a third-generation screening test for HBV in the late 1970s.[181] Today, HBV accounts for approximately 10% of posttransfusion hepatitis, a percentage that likely will decline further as vaccination becomes more widespread. The risk of transfusion-acquired infection with HBV now is estimated to be 1 in 63,000 units transfused. [205] Acute disease develops in 35% of persons infected with the virus, approximately 5% develop chronic infection, and 2% develop fulminant hepatic failure leading to death or need for a liver transplant. [202,207]

Although hepatitis A virus (HAV) can be transmitted by transfusion, more commonly it is food borne. After identification of the HAV in 1973, it became clear that a substantial proportion of new posttransfusion hepatitis cases were not caused by infection with HAV or HBV. Consequently, the term *non-A, non-B hepatitis* was coined. By the late 1970s and early 1980s, the prevalence of non-A, non-B hepatitis in multiply transfused patients was reported to be as high as 10%. [207]

Before the identification of HCV in 1988, an association between an increased alanine aminotransferase (ALT) level in donor blood and development of non-A, non-B hepatitis in recipients of that blood was observed. Surrogate testing for ALT and anti–hepatitis B core protein subsequently reduced the rate of posttransfusion hepatitis to 2% to 3% of heavily transfused recipients.[207] ontinuous refinement of HCV testing has now brought the risk of transfusion-acquired HCV to fewer than 1 in 100,000.[205] Nucleic acid testing should decrease the risk to 1 in 125,000 units. [203] The increasing sensitivity of specific HCV testing has rendered likely that surrogate ALT and hepatitis B core antibody testing, which now contribute little to identifying potentially infectious units, soon will be discontinued. [207,208]

Evidence indicates that additional viruses may account for many of the rare remaining posttransfusion hepatitis cases. HGV, which shares 25% genetic homology and coinfection with HCV, can be transmitted by transfusion.[209] Heat inactivation, as applied to plasma products to inactivate HCV, also prevents HGV transmission. [209] HGV is present in up to 5% of blood donors and in 7% to 90% of multiple transfusion recipients. [202,209]

The clinical consequences of transfusion transmission of HGV appear relatively mild, as infection does not appear to correlate with significant liver disease. [210] HGV-infected patients develop a mild hepatitis with slight ALT elevations. [211] Interestingly, coinfection with HGV does not appear to alter the course of infection with HAV, HBV, or HCV.[212] Initially, HGV was thought to be responsible for post-hepatitis aplastic anemia, but recently the virus was demonstrated to be contracted by transfusion given after affected patients became ill.[213] Currently, HGV screening of blood donors is not required. Undoubtedly, additional viruses capable of causing posttransfusion hepatitis will be identified in years to come.

#### Late Effects of Posttransfusion Hepatitis in Pediatric Cancer Patients

Because donor screening for posttransfusion hepatitis is now so effective, children currently being treated for cancer should have a low risk of acquiring hepatitis from transfusion. However, 17% to 40% of children treated a decade or more ago and cured of their cancer are chronically infected with these agents, most commonly HCV.[214,215,216 and 217] Most such patients are asymptomatic, with elevated but fluctuating ALT values. [218] Some HCV-infected cancer survivors may develop biopsy-proven cirrhosis, although this condition is more common in HBV- and HCV-coinfected individuals. [217,218] Routine follow-up of children cured of cancer, especially those who were transfused before widespread use of the third-generation HCV screening of the blood supply in 1995, should include measurement of ALT and testing for HCV infection.[218] If screening reveals a transfused cancer survivor to have persistently elevated ALT values but to be HCV-seronegative, sensitive PCR-based RNA screening for HCV is indicated.[214,215] HCV-infected patients may benefit from treatment with interferon-a, particularly in combination with ribavirin.[219,220]

Although the current risk of transfusion-associated hepatitis is low in the United States, transmission rates for HCV and other hepatotropic viruses remain markedly

higher in some areas of Western Europe and developing countries. Thus, for children with cancer treated in other locales, the risk for liver disease after treatment remains a major concern.[217,221]

### Cytomegalovirus

CMV is a cell-associated herpesvirus that causes hepatitis, an infectious mononucleosis-like illness and, in heavily immunosuppressed oncology or transplant patients, a severe, often fatal enteritis and interstitial pneumonitis. [191] CMV can be transfused by the unmanipulated leukocytes of infected donors and thus produce severe primary infection or reactivation disease in previously infected immunosuppressed patients. [191] Use of seronegative donors, removal of leukocytes by filtration, and irradiation of blood products to prevent the replication of CMV-containing leukocytes have been used to decrease the transmission risk.

The seroprevalence rate of CMV can range from 20% to 100% of blood donors, depending on geographic location. [222] Thus, finding adequate numbers of serologically negative donors to exclusively supply blood product needs is difficult. The 3-log reduction of white cells accomplished with the use of leukocyte depletion filters (discussed in the section Leukocyte Depletion) has been considered as effective as the exclusive use of seronegative blood in preventing transfusion transmission of CMV, even to ransplant recipients.[223]

However, debate continues over a possible small benefit from the use of seronegative rather than filtered blood products. [202,224] Cell-free samples from seropositive blood units have been demonstrated after PCR amplification to contain low levels of infectious CMV. [225] Not clear is whether the virus originated from leukocytes during storage or whether CMV already was present in the plasma while all leukocytes were intact. [202] Further studies will be necessary to determine the magnitude of this risk in transfusion recipients.

### Parvovirus B19

The nonenveloped parvovirus B19 may result in a persistent infection leading to prolonged erythroblastopenia or pancytopenia in immunocompromised patients with leukemia, prior renal transplantation, or acquired immunodeficiency syndrome. [5,6 and 7,202] Infection via transfusion has been documented but is rare. [5] Parvovirus B19 is implicated also in postinfection arthropathy and vasculitic phenomena in immunologically normal individuals. [5] Some estimates maintain that 0.03% to 0.60% of blood donors are viremic with B19.[226] However, donor screening must be PCR-based because seroconversion does not correlate with infectivity. [5] At present, no such screening is implemented or planned, so parvovirus B19 transmission by transfusion continues to exist. [202]

### Creutzfeldt-Jakob Disease

Creutzfeldt-Jakob disease (CJD) is a neurodegenerative disease, the human form of spongiform encephalopathy. Most likely it is transmitted by an infectious prion. CJD exists in forms that are familial (10% to 15% of cases), sporadic (85% to 90% of cases), and acquired (1% of cases). Acquisition is from transplantation of dura mater or use of cadaveric human growth hormone. A theoretical risk of transmission by blood products exists, and animals inoculated intracerebrally with blood from CJD patients have developed a similar disease. [202,227] The FDA has mandated the withdrawal of blood products from persons in whom CJD develops and suggests notification of recipients. In some centers, the withdrawal and look-back notification are applied to blood products from relatives of CJD cases as well. However, nothing can be done to prevent the disease, and physicians can only offer reassurance that the risk is at this time only theoretical and not documented. [227]

#### *Other Complications of Transfusion*

### Hemochromatosis

Transfusion-related hemochromatosis or iron overload is of concern in intensively treated or transplanted survivors of childhood cancer. [228] Iron-induced injury may potentiate the hepatic damage of viral infection or chemotherapy. If erythrocyte transfusion volume was substantial during therapy, screening of childhood cancer survivors by serum ferritin or transferrin saturation is indicated. If these screening tests suggest iron overload, determination of hepatic iron concentration by biopsy followed by phlebotomy or iron chelation therapy, if indicated, must be undertaken. The management of iron overload is beyond the scope of this chapter but is reviewed elsewhere.[229]

### Immunomodulation and Enhancement of Tumor Growth

Immunomodulation from allogeneic blood transfusion is important in improving renal allograft survival and in decreasing recurrent spontaneous abortion. [230,231] Although the mechanisms and mediators of this immunosuppressive effect are beyond the scope of this chapter, this condition appears to be induced by a recipient's response to the transfusion of allogeneic leukocytes. [231,232] Adult surgical patients appear to have an increased risk of postoperative infection if allogeneic transfusion is required. Whether this is due to clinical confounders, such as volume of blood loss, or to duration of the operation is unclear. [232,233] A large number of conflicting studies further suggest that the “allogeneic transfusion effect” may promote the growth of adult surgically treated cancers, such as colon or breast carcinomas, sarcomas, and non-Hodgkin's lymphoma.[233] Prestorage leukoreduction appears effective in ameliorating these effects. [185,231] No convincing data confirm that allogeneic blood transfusion enhances neoplastic growth or negatively influences disease-free survival in pediatric malignancies. [228]

## WHITE BLOOD CELLS

Opportunistic infections associated with prolonged periods of severe neutropenia (i.e., gram-positive and gram-negative bacteria and fungal pathogens) continue to be a major source of morbidity and mortality in children undergoing myelosuppressive chemotherapy. Improvements in supportive care, with broad-spectrum antibiotics and hematopoietic CSFs such as G-CSF and GM-CSF, have allowed for the administration of higher doses of conventional chemotherapy and myeloablative regimens followed by autologous or allogeneic stem-cell support. This increased dose intensity has led to improved disease-free survival for certain pediatric malignancies. As the dose intensity of chemotherapeutic regimens continues to rise, the depth and duration of chemotherapy-induced neutropenia—defined as an absolute neutrophil count (ANC) of less than 500 cells per $mm^3$—also will increase.

### Pathophysiology

#### *Decreased Numbers*

The most important risk factor for development of serious infection in a cancer patient with newly diagnosed disease or during myelosuppressive chemotherapy is the severity and duration of neutropenia. [234] Decreased numbers of neutrophils result from the underlying malignancy or are secondary to treatment. Prolonged neutropenia is an important risk factor in the development of infection, especially with fungal pathogens, such as *Candida* and *Aspergillus* species. The rate of decrease in the number of neutrophils also may be associated with an increased risk of infection. [234]

#### *Decreased Function*

Qualitative defects of neutrophil function are common in cancer patients. A number of descriptive studies have demonstrated varying degrees of impaired function in neutrophils isolated from patients undergoing chemotherapy. Documented defects include impairment of superoxide generation, phagocytosis, and microbicidal activity *in vitro*. These defects may be due to the underlying malignancy, chemotherapeutic agents, radiation therapy, or the presence of viral infections, such as CMV.[234]

### Granulocyte Transfusions

Attempts to prevent and treat infections with granulocyte infusions have been inconclusive. Wide interest was seen in the use of granulocyte transfusions in the 1970s and 1980s, and a series of clinical studies performed at that time demonstrated moderate success in certain clinical situations. However, these studies were hampered by infusion of relatively low numbers of neutrophils and the possible development of alloimmunization owing to the development of antineutrophil antibodies.[235] Even at present, the requirement that infusion of granulocytes be undertaken shortly after collection, development of leukocyte incompatibility,

questionable clinical efficacy, and occasional adverse reactions (e.g., CMV infection, pulmonary infiltrates) have resulted in infrequent use of therapeutic granulocyte infusions for neutropenic cancer patients.

Opportunistic infections, especially due to fungal pathogens, continue to be a source of morbidity and mortality despite the use of CSFs and antifungal agents. The superior collection techniques available today and the use of CSFs or steroids to stimulate donor neutrophil counts prior to collection allow collection of larger numbers of granulocytes. Therefore, granulocyte transfusion has once again become an area of clinical research.

### *Granulocyte Collection and Infusion*

Granulocytes are collected via apheresis using a specialized centrifuge. [235] Cells are separated on the basis of density, and the erythrocytes and platelets are returned to the donor. Granulocyte collections taken by centrifugation from healthy, unstimulated donors usually yield between 0.5 and 1.0 × $10^{10}$ cells per liter of donor blood. The addition of rouleaux-inducing agents, such as hydroxyethyl starch, pentastarch, or dextran, can enhance the sedimentation of red cells from the granulocytes and increase yield.[235] Donor granulocyte counts also are increased by the administration of corticosteroids or CSFs that mobilize granulocytes from the marginating pool into the circulating pool or increase their production. [236,237,238 and 239] Granulocytes always are irradiated prior to infusion to prevent transfusion-related GVHD. [235]

### *Role of Hematopoietic Growth Factors in Granulocyte Collection*

With the advent of hematopoietic growth factors, interest in the use of granulocyte infusions has increased. The number of granulocytes infused plays an important role in efficacy.[240] G-CSF priming of donors has been shown to increase the yield of neutrophils during apheresis. [241] G-CSF given every other day is equivalent to daily G-CSF dosing and is superior to prednisone alone to increase the number of granulocytes collected. [237] Clinical trials are under way to study the efficacy of G-CSF-stimulated granulocytes in the prevention and treatment of infections in neutropenic cancer patients.

## Clinical Use of Granulocyte Transfusions

### *Infection in Neutropenic Patients*

The optimal use of granulocyte infusion in the treatment of infection in neutropenic patients is unknown. Randomized studies of the efficacy of granulocyte transfusions given with intravenous antibiotics have been reported, but interpretation of these studies is difficult, as the number of granulocytes infused is relatively small.[235] Alloimmunization with antineutrophil antibodies also may be a substantial barrier. [242]

Few studies have examined the role of G-CSF–primed neutrophils in the prophylaxis and treatment of bacterial or fungal infections. Selection of patients is difficult, but criteria should include an ANC of fewer than 500 cells per $mm^3$, persistent documented bacterial or fungal infection despite adequate antibiotic or antifungal therapy, and no evidence of marrow recovery.

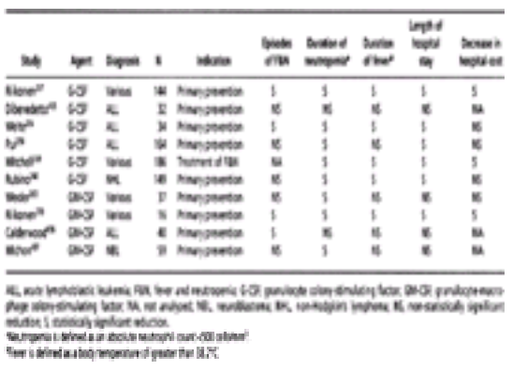
### *Adverse Events Related to Granulocyte Transfusions*

Transfusions of granulocytes in patients who have been alloimmunized to either HLA or neutrophil-specific antigens can result in fever and occasionally acute pulmonary symptoms, including chills, dyspnea, development of infiltrates seen on chest radiograph, chest tightness, acral cyanosis, and hypoxia. These episodes may take up to 12 hours to resolve. Concurrent administration of amphotericin B and granulocyte concentrates has been reported to cause particularly severe pulmonary reactions.[243] Separation of the two infusions by a minimum of 4 hours should circumvent this problem. [235]

## HEMATOPOIETIC COLONY-STIMULATING FACTORS

Improved survival in pediatric malignancies is due in large part to intensive chemotherapeutic regimens that now are better tolerated owing to improvements in supportive care. Children receiving high-dose chemotherapy are at increased risk of developing potentially life-threatening infections during periods of chemotherapy-induced neutropenia. The incidence and severity of these infections are related to the duration and severity of neutropenia. Methods used to prevent infection have included delay or reduction in chemotherapy dose, progenitor cell support, and use of prophylactic antibiotics. During the last decade, CSFs have been introduced into clinical practice, and the prophylactic use of these agents has been demonstrated to reduce the duration of neutropenia and the number of infections and hospitalizations.[244] CSFs usually are begun on the day after chemotherapy, but several recent studies have shown that CSF treatment may be delayed safely for several days after completion of a course of chemotherapy and can yield equivalent results. [244]

Although clinical benefit has been documented, the high cost of CSFs has led to concerns about their appropriate use. The American Society of Clinical Oncology (ASCO) has developed guidelines for the use of CSFs based on an expert multidisciplinary panel review of the available clinical data. Originally published in 1994 and updated in 1996, the most recent version was released in 2000. [245,246 and 247] In all versions, the panel recommended the prophylactic use of CSFs by patients in whom the expected incidence of chemotherapy-induced neutropenia is greater than or equal to 40%, after a prior episode of febrile neutropenia, or after high-dose chemotherapy with autologous progenitor stem-cell support. CSFs were found to be beneficial also in the mobilization of peripheral blood progenitor cells (PBPCs). The panel stated that although pediatric data are not conclusive, the guidelines recommended for adult patients generally are applicable to children as well. [246,247] A review of CSF use practices by members of the Pediatric Oncology Group indicates that pediatric oncologists use CSFs more commonly than do their adult colleagues and initial ASCO guidelines recommended. [248] Although much of the CSF use was dictated by protocol requirements, it was influenced also by physician preference and available data.[247] The higher perceived dose intensity and expected toxicity of pediatric chemotherapeutic regimens also appear to promote CSF use.[248] The 2000 ASCO guidelines make no new pediatric recommendations.[247] Further research is required to define more clearly the role of CSFs in children receiving chemotherapy and progenitor-cell transplantation. The results of selected randomized trials of white cell growth factors in pediatric patients are summarized in Table 40-6.



| Study | Agent | Diagnosis | N | Indication | Episodes of FEVA | Duration of neutropenia[a] | Duration of fever[b] | Length of hospital stay | Decrease in hospital cost |
|---|---|---|---|---|---|---|---|---|---|
| Kilmer[237] | G-CSF | Various | 144 | Primary prevention | S | S | S | S | S |
| DiBenedetto[238] | G-CSF | ALL | 32 | Primary prevention | NS | NS | NS | NS | NA |
| Meta[239] | G-CSF | ALL | 34 | Primary prevention | S | S | S | S | NS |
| Ky[240] | G-CSF | ALL | 104 | Primary prevention | NS | S | NS | S | NS |
| Kilmer[241] | GM-CSF | Various | 74 | Primary prevention | S | S | S | S | S |
| Calderwood[242] | GM-CSF | ALL | 40 | Primary prevention | S | NS | NS | NS | NA |
| Mitchell[243] | GM-CSF | NBL | 59 | Primary prevention | NS | S | NS | NS | NA |

ALL, acute lymphoblastic leukemia; FEVA, fever and neutropenia; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; NA, not analyzed; NBL, neuroblastoma; NHL, non-Hodgkin's lymphoma; NS, non-statistically significant reduction; S, statistically significant reduction.
[a]Neutropenia is defined as an absolute neutrophil count <500 cells/mm³.
[b]Fever is defined as a body temperature of greater than 38.3°C.

**TABLE 40-6. SUMMARY OF SELECTED RANDOMIZED TRIALS OF HEMATOPOIETIC GROWTH FACTORS IN PEDIATRIC PATIENTS**

### *Molecular and Cellular Physiology of Growth Factors*

Homeostatic mechanisms maintain the number of red blood cells, platelets, and mature myeloid and monocytic cells within a narrow range. Reductions in the numbers of these cells (e.g., as a result of chemotherapy) activate physiologic mechanisms that attempt to normalize the blood components. The CSFs can be divided into those that expand early hematopoietic progenitor cells (IL-3, IL-6, IL-11, stem-cell factor, and flt3 ligand) and those that are late-acting with biologic effects primarily limited to a single hematopoietic lineage (G-CSF, GM-CSF, and erythropoietin). However, the late-acting growth factors also may influence more than one hematopoietic lineage.[244]

### *Homeostatic Mechanisms*

Under normal conditions, serum levels of CSFs are fairly low, but they can increase markedly in response to specific stimuli, such as periods of stress (e.g., infection) and when the terminally differentiated cells regulated by the CSFs are substantially reduced. [244] Serum G-CSF levels appear to be controlled by changes in both the rate of G-CSF production and clearance. Lipopolysaccharides produced by pathogenic bacteria increase the rate of synthesis of G-CSF and other CSFs. Although normal serum G-CSF levels are approximately 25 pg per mL, concentrations of 1,000 pg per mL or higher can be observed in patients with severe infections [249] and after stem-cell transplantation.[250,251] Conversely, high neutrophil levels appear to increase CSF clearance. [252,253]

## Clinical Applications of Growth Factors

Three basic strategies are applied in the use of CSFs in the management of patients with malignancy. Primary prophylaxis is defined as the administration of a CSF in an attempt to prevent myelosuppression. Secondary prophylaxis involves the use of a CSF to prevent new episodes of myelosuppression or delays in the administration of subsequent cycles of chemotherapy in a patient who previously has experienced delays in therapy. CSF treatment is the use of CSFs to shorten the duration of an already established episode of neutropenia prior to the development of fever, as a component of the treatment of an episode of febrile neutropenia, or after a documented infectious complication has occurred.

### *Primary Prophylaxis in Patients after Chemotherapy*

#### Granulocyte Colony-Stimulating Factor

A variety of phase I and phase II studies in children having cancer and receiving intensive chemotherapy have documented that primary administration of G-CSF reduces the duration of neutropenia, lowers rates of neutropenic fever, decreases use of antibiotics, and diminishes the need for hospitalization as compared with historical control subjects who did not receive G-CSF. [244,254,255] Several larger randomized trials also have been completed in children having cancer and undergoing chemotherapy. Children with high-risk leukemia randomly assigned to receive G-CSF had a lower incidence of febrile neutropenia and culture-confirmed infection and a shorter duration of total antibiotic use as compared with those receiving placebo. [256] Another trial in children undergoing chemotherapy showed a reduction in the duration of neutropenia (4.8 days vs. 16.5 days), days of hospitalization (13 days vs. 65 days), and broad-spectrum antibiotic treatment (13 days vs. 95 days) for patients receiving G-CSF as compared to those receiving placebo. Two episodes of neutropenic fever occurred in patients in the G-CSF group, and ten took place in those in the placebo group.[257]

However, not all studies have shown a benefit from the use of G-CSF. In 164 children with acute lymphoblastic leukemia undergoing continuation chemotherapy and randomly assigned to receive either G-CSF or placebo, no significant difference was noted between the two groups in rate of hospitalization for febrile neutropenia (58% in the G-CSF group vs. 68% in the placebo group), event-free survival at 3 years (83% in both groups), or number of severe infections (five in patients in the G-CSF group vs. six in those in the placebo group). Patients treated with G-CSF did have shorter median hospital stays (6 days vs. 10 days) and fewer documented infections (12 vs. 27). The median total cost of supportive care was similar in those in the G-CSF and placebo-treated groups. [258] A Pediatric Oncology Group study also showed no significant benefit in a randomized trial of G-CSF versus placebo in children with T-cell leukemia and advanced-stage lymphoblastic lymphoma. [259] Another trial in 149 children and adolescents receiving chemotherapy for non-Hodgkin's lymphoma also showed no significant differences between the two groups in terms of febrile neutropenia, duration of neutropenia, and total costs of treatment. [260]

A beneficial effect of G-CSF on platelet levels has not been observed. In fact, several trials have shown that G-CSF-treated patients develop somewhat more severe thrombocytopenia in later cycles, perhaps as a result of higher chemotherapy dose intensity. [244] Amelioration of mucositis in association with G-CSF has been reported.[87] Conversely, in a trial of weekly chemotherapy for non-Hodgkin's lymphoma, patients who received G-CSF had more treatment delays for mucositis than did control subjects. This probably occurred because the G-CSF treated patients received more intensive treatment over the entire course of chemotherapy as a result of the lesser prevalence of neutropenia.[261] In conclusion, it appears that the use of G-CSF should be limited to pediatric patients who receive intensive chemotherapeutic regimens, as recommended in the ASCO guidelines. For malignancies in which available data do not demonstrate an improvement in survival with increasing dose intensity, consideration should be made to reduce chemotherapy doses rather then using a CSF. [247] The routine use of these costly agents after pulses of less myelosuppressive chemotherapy cannot be supported by the literature.

#### Granulocyte-Macrophage Colony-Stimulating Factor

Randomized trials of GM-CSF as primary prophylaxis of neutropenic fever usually have resulted in lessened duration or severity of neutropenia, but clinical benefits have not been as consistently positive as those observed with G-CSF. [244,255,262] Two prospective studies of children have been reported. In the first, GM-CSF prophylaxis was begun during the third treatment cycle of alternating vincristine, doxorubicin, and cyclophosphamide alternating with and ifosfamide plus etoposide for pediatric sarcomas.[263] Although GM-CSF reduced the depth and duration of the neutropenic nadir, the rates of neutropenic fever, hospital days, antibiotic requirements, dose intensity, and disease control were not affected significantly. Moreover, thrombocytopenia was more severe in children receiving GM-CSF, resulting in greater platelet and red blood cell transfusion requirements. [263] The other randomized trial of GM-CSF in children also documented a shortened duration of neutropenia for children receiving GM-CSF, but the period of thrombocytopenia was longer. [264]

### *Secondary Prophylaxis in Patients after Chemotherapy*

Currently, no randomized trials are assessing whether secondary prophylactic use of either G-CSF or GM-CSF can prevent new episodes of febrile neutropenia in pediatric patients with a prior episode, and encouraging results in adults are limited. In a randomized trial of G-CSF versus placebo in adults, patients on the placebo arm were crossed over to G-CSF during subsequent cycles if they had a fever in cycle 1. Patients receiving G-CSF had a shorter duration of neutropenia (median of 6.0 days vs. 2.5 days) and a reduction in the rate of neutropenic fever (100% vs. 23% after receiving G-CSF). Patients who continued to receive placebo had a median duration of neutropenia of 6 days, although the incidence of febrile neutropenia in the second cycle was only 5%. [265] Therefore, the routine use of G-CSF for secondary prophylaxis cannot be recommended.

### *Colony-Stimulating Factors as Therapy for Neutropenia*

CSFs sometimes are administered concomitantly with intravenous antibiotics in patients with febrile neutropenia. As the response rate of these patients to antibiotic therapy is high, the likelihood of detecting a therapeutic benefit of CSFs is minimal. The utility of CSF therapy, therefore, may have to be gauged in terms of improved quality of life using pediatric-specific quality-of-life instruments, indirectly by duration of hospital stay and antibiotic use, or reduced treatment costs.

A series of studies have examined the efficacy of initiating CSF therapy after neutropenia is identified during a chemotherapy cycle. Two relatively small, randomized trials failed to detect a clinical benefit for either G-CSF [266] or GM-CSF[91] in this setting. Another randomized study assigning afebrile neutropenic patients to either G-CSF or placebo showed that the median time to an ANC greater than 500 cells per $mm^3$ was significantly shorter for patients who received G-CSF (2 days vs. 4 days). In this study, no effect of G-CSF was seen on the rate of hospitalization, number of days in the hospital, duration of treatment with antibiotics, or number of culture-positive infections.[267] Therefore, administering CSFs to afebrile patients with neutropenia is unlikely to prove clinically valuable.

Several randomized controlled trials have evaluated either G-CSF, GM-CSF, or a combination of both agents as adjunctive treatment for patients with chemotherapy-induced neutropenia who already have fever.[244,255,262] In the largest of these trials, 216 patients were enrolled. Although median ANC recovery was statistically more rapid in patients in the G-CSF-treated group (3 days vs. 4 days), no significant difference was noted between the two groups as regards more clinically relevant parameters of fever duration, days of antibiotic use, duration of hospital stay, and mortality due to infection. [268]

In a double-blind study, pediatric patients who had neutropenia (ANC fewer than 500 cells per $mm^3$) and developed fever were randomly assigned to receive G-CSF (5 µg per kg per day) or placebo in addition to intravenous antibiotic therapy. Children randomly assigned to G-CSF had a shorter median hospital stay (5 days vs. 7 days) and fewer days of antibiotic use (5 days vs. 6 days) than did those receiving placebo. The 2-day reduction in hospital stay reduced the median cost by 29% per admission.[269] In a randomized study of 58 episodes of chemotherapy-induced febrile neutropenia in 40 children, the group given 5 µg per kg per day of GM-CSF

instead of placebo had a comparable slight reduction in hospital stay (9 days vs. 10 days) and antibiotic usage (7.0 days vs. 8.5 days). [270]

Other clinical trials evaluating CSF treatment for febrile patients with neutropenia have produced variable results, probably because of differences in patient characteristics, study design, and data analysis. In a randomized study of GM-CSF versus placebo in 107 adults, GM-CSF improved the response rate in terms of defervescence but not overall survival.[244] In a pediatric trial, GM-CSF therapy for febrile neutropenia after intensive chemotherapy shortened the mean duration of severe neutropenia from 9 days in the placebo group to 7 days in the GM-CSF treatment group. [263,271]

The ASCO guidelines state that because of the limited data regarding the efficacy of CSFs in afebrile patients with neutropenia, the use of CSFs in these patients is not recommended.[247] For febrile neutropenic patients as well, the routine initiation of CSF as an adjunct to antibiotic therapy is unnecessary. However, continuing G-CSF if started before the onset of febrile neutropenia is certainly warranted.

### *Chemotherapy Dose Intensity*

A number of preclinical studies and retrospective analyses have correlated chemotherapeutic efficacy with the dose intensity of the chemotherapy administered. Higher rates of response to antineoplastic agents have been related to delivery of full-dose chemotherapy combinations [272,273 and 274] or to overall dose intensity. Early phase I and phase II trials of both G-CSF and GM-CSF indicated that improvement in on-time, full-dose chemotherapy delivery was achieved with their use. [275,276] These results suggested that CSF prophylaxis may allow for increases in the dose intensity of chemotherapy, with consequent improvements in the antineoplastic effects of the cytotoxic drugs. A number of pediatric studies have evaluated chemotherapeutic regimens in which the doses of agents are escalated beyond those that can be given on time and with an acceptable incidence of febrile neutropenia without prophylactic use of CSFs. Regimens using even higher doses of cyclophosphamide (e.g., more than 4.0 g per $m^2$ per course) have been evaluated for neuroblastoma,[277] Ewing's sarcoma,[278] and other pediatric solid tumors.[279]

Another strategy for increasing the dose intensity of cytotoxic chemotherapy is to compress the interval between chemotherapy courses with CSF support. A randomized study of children with acute myelogenous leukemia demonstrated the efficacy of this approach.[280] Trials in adults employing the approach of rapid delivery of a moderately intensive chemotherapeutic regimen also have been successful in achieving enhanced chemotherapy delivery. [89,281,282,283,284 and 285]

Randomized trials of adult patients have shown no benefit in overall survival or disease-free survival for most tumors when the dose of chemotherapy was maintained and secondary prophylaxis with myeloid CSFs was instituted. The 2000 ASCO guidelines recommend that except for curable tumors or tumors for which data exist to maintain dose intensity, chemotherapy dose reduction should be considered after neutropenic fevers occur or severe or prolonged neutropenia is experienced after prior cycles.[247]

Many pediatric tumors are curable in a significant percentage of patients; accordingly, pediatric oncologists in practice are reluctant to reduce dose intensity as an alternative to CSF use. Indeed, in the Pediatric Oncology Group survey, chemotherapy dose reduction alone never was selected as a strategy to prevent febrile neutropenia for pediatric patients.[248] The ASCO guidelines recommend that use of CSFs to maintain dose intensity should be limited to clinical research protocols. [247]

### *Autologous Bone Marrow and Peripheral Progenitor Stem-Cell Rescue*

Randomized studies have evaluated the use of CSFs after autologous bone marrow rescue.[244,255,262] In general, neutrophil recovery was significantly hastened for patients receiving CSFs. One study demonstrated that G-CSF support resulted in a reduction of duration of grade 4 neutropenia, from a median of 20 to 13 days. [286] Randomized trials of G-CSF or GM-CSF prophylaxis after autologous BMT demonstrated significant effects on clinically relevant end points, such as duration of antibiotic usage and days in the hospital. In these trials, G-CSF use was associated with a significant reduction in duration of fever, a result that was not seen with GM-CSF use.[287]

Several small, retrospective studies of PBPC transplantation, including one study of children, found no significant benefit for postinfusion G-CSF in terms of either neutrophil or platelet recovery, raising the possibility that a larger number of infused progenitor cells obviates the beneficial effects of CSF therapy. [288,289] Randomized clinical trials have demonstrated a modest clinical benefit for G-CSF alone and for G-CSF plus GM-CSF in terms of more rapid neutrophil engraftment and shorter duration of hospitalization.[290]

On the basis of these data, the ASCO guidelines state that CSFs can successfully shorten the period of neutropenia and reduce infectious complications in patients undergoing high-dose cytotoxic therapy with autologous BMT.[247] Therefore, use of CSFs in this setting appears to be reasonable.

### *Allogeneic Bone Marrow Transplantation*

Neutrophil recovery usually has been enhanced with the use of either G-CSF or GM-CSF after allogeneic BMT. [244] No evident increase has been seen in GVHD, graft rejection, or relapse associated with CSF use in this setting. [286,291,292,293 and 294] Several studies have suggested a trend in favor of enhanced disease-free survival in CSF-treated patients. However, duration of hospitalization has not been altered significantly by administration of CSFs after allogeneic BMT. [292,293 and 294]

Pediatric data regarding the use of CSF prophylaxis after allogeneic BMT consist only of phase II trials compared to historical controls. In a small study, karyotypic analysis of recipient leukocytes in patients undergoing BMT from a donor of the opposite gender indicated that G-CSF allowed detection of donor karyotype 8 days faster than in controls.[295]

Based on this experience, the ASCO guidelines suggest a potential benefit to patients undergoing allogeneic BMT, but these data remain less conclusive than those for patients undergoing autologous BMT.[247] Therefore, routine use of CSFs after allogeneic transplantation cannot be recommended at the present time.

### *Engraftment Failure after Bone Marrow Transplantation*

In some patients who undergo high-dose therapy followed by stem-cell rescue, neutrophil engraftment does not occur, is delayed, or is lost after return of granulocytopoiesis. Patients who received T-cell–depleted grafts, whose donors are an HLA-C mismatch, whose graft is purged *in vitro* with chemotherapy, or who receive a low cell dose per kg of recipient body weight (e.g., umbilical cord blood graft recipients) are at particularly increased risk of primary or secondary graft failure. Mortality from infection in these patients is substantial, and this has been the impetus for studies of CSFs as a possible means to ameliorate this complication.

Most trials have evaluated the use of GM-CSF in patients who did not receive primary prophylaxis with a CSF and who failed to recover neutrophil counts by 3 to 4 weeks after autologous BMT. The largest of these studies demonstrated a significant reduction in death rates associated with the use of GM-CSF. [296] This study and other smaller trials have demonstrated neutrophil responses to GM-CSF administration in approximately one-half to two-thirds of such patients. However, interpreting these studies is difficult. Comparisons with historical controls, particularly in evaluating indirect end points, such as infectious mortality, have the strong potential for patient selection bias. Descriptive accounts of neutrophil recovery associated with CSF use are also uncertain. Quantifying the incremental benefit associated with GM-CSF use in these patients is rendered difficult by the extremely variable rate of spontaneous neutrophil recovery. On the basis of the existing data, the ASCO guidelines state that CSFs may have a role in assisting in the recovery of patients who experience delayed or inadequate neutrophil engraftment after progenitor cell transplantation.[247]

### *Mobilization and Collection of Peripheral Blood Progenitor Cells*

The collection and infusion of PBPCs as a means of hastening hematopoietic recovery after myeloablative chemotherapy are described in more detail in Chapter 16. Increasing experience is being gained in the use of PBPCs in the allogeneic setting, although they are used primarily for autologous transplant. PBPCs are collected by means of leukapheresis, are stored, and then are infused after the administration of myeloablative therapy. A total of 2.5 to 5.0 × $10^6$ $CD34^+$ cells per kg of recipient body weight usually are collected. Harvesting PBPCs without the use of chemotherapy or CSF priming requires multiple pheresis collections, as only small numbers of progenitors normally are present in the circulation. [297] Administration of nonablative chemotherapy with such agents as cyclophosphamide was found to cause a large increase in circulating PBPC numbers during hematologic recovery. Both GM-CSF and G-CSF stimulate release of PBPCs from the bone marrow into the peripheral blood. Peak yields usually are observed 4 to 8 days after CSF treatment alone, whereas peak PBPC numbers after administration of both

chemotherapy and CSF usually are noted shortly after recovery from neutropenia. A number of large historically or sequentially controlled trials provide convincing evidence that inclusion of CSFs in the mobilization regimen allows for collection of more PBPCs than are obtained from unstimulated donors or after hematologic recovery from chemotherapy alone.[244,255,262] In one randomized trial, 22 patients were assigned to receive or not receive G-CSF. Patients receiving G-CSF had a 2.5- to 5.5-fold increase in the numbers of mononuclear cells and PBPCs in their leukapheresis products. [298] Evidence also substantiates that CSF-assisted mobilization of PBPCs may reduce harvesting and posttransplantation supportive care costs. A randomized trial comparing the efficacy of different CSFs (G-CSF, GM-CSF, G-CSF combined with GM-CSF, and PIXY-321) in mobilizing PBPCs showed no difference between the various mobilization agents. The ASCO guidelines recommend the use of CSFs in the collection of PBPC.[247] The optimal dose of G-CSF with or without chemotherapy is under investigation, but a higher dose of G-CSF (10 µg per kg per day) may yield a greater content of $CD34^+$ progenitor cells in the PBSC product.

CSF-mobilized PBPCs are being evaluated also for use in the allogeneic transplantation setting. Potential advantages to the donor include less discomfort during the procedure and, in most cases, avoidance of general anesthesia.[299] Concerns have been raised about the use of CSFs in otherwise healthy donors, owing to the possible risks of secondary malignancies that have been associated with their use in congenital neutropenia, aplastic anemia, and other nonmalignant conditions (discussed in the section Potential of Colony-Stimulating Factors to Induce Malignancy ). Recipient benefits include faster hematopoietic reconstitution after transplantation and a reduction in the morbidity and mortality of the procedure. G-CSF-primed leukocytes might theoretically carry a greater risk of acute or chronic GVHD, as patients receiving PBPC would receive a dose of mature lymphocytes higher than that given with bone marrow. However, early experience suggests that CSF-mobilized PBPCs may provide rapid hematologic recovery without an appreciably greater incidence of GVHD. [299,300 and 301]

In children undergoing PBPC transplantation, pediatric-specific problems can be encountered. Unlike adults in whom large, peripheral intravenous catheters can be placed for PBPC collection, most children require a rigid central venous catheter to be placed to collect an adequate number of stem cells. Permanent, indwelling pheresis catheters can be placed at the beginning of therapy, thus avoiding surgery for the placement of a second central venous catheter, if transplantation is an option early in the treatment course. Other potential complications include the development of thrombocytopenia, [302] hypocalcemia secondary to the large volume of citrate anticoagulant in the circuit,[303] headache, nausea, and vomiting. Techniques for minimizing or managing these complications have been developed, and PBPC harvesting now is considered safe and feasible for infants and young children whose weight is more than 10 kg. [304] CSFs used in the harvesting procedure may alleviate some of the difficulties of collection in younger donors by reducing the number of pheresis collections necessary to harvest an adequate amount of PBPC for transplantation.[305,306]

### *Colony-Stimulating Factors as Antitumor Therapy*

#### Priming Effect of Granulocyte-Macrophage Colony-Stimulating Factor before or during Chemotherapy for Acute Myeloid Leukemia

*In vitro* evidence exists that myeloid leukemic cells have receptors for GM-CSF and that their proliferation and differentiation are supported by exposure to GM-CSF.[262] Recruitment of chemoresistant cells into more sensitive phases of the cell cycle was hypothesized to enhance the effect of chemotherapeutic agents. GM-CSF has been shown to increase the number of leukemia cells in S phase. In a randomized trial of 114 patients with newly diagnosed AML, patients were randomly assigned to receive chemotherapy alone or with GM-CSF starting 24 hours beforehand. [307] The overall remission rate was 79% in patients receiving GM-CSF, compared to 84% in controls. Similar studies in patients with AML showed a trend toward increase in disease-free survival; however, the use of GM-CSF during induction therapy of AML did not appear to have a significant impact on treatment outcome. [308]

#### Granulocyte-Macrophage Colony-Stimulating Factor as an Adjunct to Antitumor Vaccine Therapy

GM-CSF might be expected to enhance antitumor vaccine immunogenicity on the basis of its role as a mediator of proliferation, maturation, and migration of dendritic cells as well as induction of primary and secondary T-cell responses. [309,310 and 311] GM-CSF was administered as an adjunct to a melanoma vaccine in 20 patients with stage IV melanoma. Four patients had a complete or partial response, and four had stable disease. [312] GM-CSF isbeing investigated also as an adjunct to tumor vaccines in multiple myeloma.[313,314]

#### Granulocyte-Macrophage Colony-Stimulating Factor as an Adjunct to Antitumor Immunotherapy

GM-CSF has been shown to enhance slightly the cytotoxic activity of peripheral blood monocytes and lymphoctyes and to increase antibody-dependent cellular cytotoxicity.[315,316] The effect of GM-CSF on activated killer T-cell activity was evaluated in 20 patients with AML and undergoing autologous BMT. [317] In this study, activated killer cell function was enhanced by GM-CSF *in vitro*. The actuarial rate of relapse in the GM-CSF-treated group was 37.4%, as compared with 49.5% in controls ($p$ = .05). Twenty-four patients with metastatic renal cell carcinoma were treated with GM-CSF; one patient had stable disease, and the rest progressed. [318] GM-CSF has been studied also in combination immunotherapy. In one trial, GM-CSF and IL-2 were administered to 20 patients with metastatic renal cell carcinoma. No responses occurred among the 20 patients, although 1 had a partial response and 3 had stable disease. [319] In a trial of 20 patients with melanoma, GM-CSF was administered as adjuvant therapy with R24, a murine monoclonal antibody that mediates complement-dependent cellular cytotoxicity of melanoma tumor targets. Of six patients who received GM-CSF alone, three had no response, and three had progressive disease. Of the 14 patients who received GM-CSF and R24, 2 had a partial response.[320] In a phase II study of GM-CSF and chimeric anti-GD2 monoclonal antibody in patients with multiply recurrent neuroblastoma, 1 of 27 patients had a complete response, 3 had a partial response, and 2 had stable disease. [321] Randomized studies are required to determine whether GM-CSF is efficacious as an adjunct to antitumor immunotherapy.

### *Hematologic Disorders and Premalignant Disorders*

#### Myelodysplasia

CSFs have been evaluated in treating patients with refractory anemia, refractory anemia with an excess of blasts, refractory anemia with an excess of blasts in transformation, and chronic myelomonocytic leukemia.[262] However, the application of CSFs has been limited by theoretic concerns about their ability to stimulate the growth of myeloid leukemias (discussed in section Potential of Colony-Stimulating Factors to Induce Malignancy ). In five separate clinical trials, 38 of 45 patients who had myelodysplasia (MDS) and received GM-CSF had an improvement in their neutrophil counts. [322] In a randomized trial, 133 patients with MDS were randomly assigned to receive GM-CSF or placebo. GM-CSF use was associated with a significant reduction in major infections in association with increases in ANC. G-CSF likewise has been evaluated in patients with MDS. [323] G-CSF appears to cause more differentiation and less proliferation than does GM-CSF. In a large randomized trial in which 102 patients received G-CSF or placebo, G-CSF was associated with an increase in ANC and a decrease in infectious complications. [324]

#### Aplastic Anemia

Aplastic anemia is a heterogeneous disorder caused by absent or defective stem cells, microenvironmental defects, or immunologically mediated bone marrow suppression. CSFs have been investigated in the treatment of aplastic anemia, often in combination with immunosuppressive agents such as cyclosporine and antithymocyte globulin. Several case series have shown that CSFs alone may induce a complete or partial response in some patients. Patients with a baseline ANC of more than 200 cells per $mm^3$ appear to have a response rate to CSFs superior to that in patients with a lower ANC. [244]

#### Kostmann's Syndrome

Severe congenital neutropenia, or Kostmann's syndrome, is a disorder of impaired neutrophil differentiation and baseline neutrophil counts of fewer than 200 cells per $mm^3$. Bone marrow examination shows a maturation arrest at the promyelocyte stage.[325] Serum levels of G-CSF in such patients are normal or elevated. Several studies have demonstrated a remarkable increase in the neutrophil counts of almost all patients treated with G-CSF. The doses required to keep the ANC above 1,000 cells per $mm^3$ varied from 3 to 15 µg per kg per day or more of G-CSF.[326] A multicenter phase III study of G-CSF in 120 patients with severe chronic neutropenia, including Kostmann's syndrome, Shwachman-Diamond syndrome, and myelokathexis reported complete responses in 108 patients, partial responses in 4, and failure to respond in only 8 patients.[327]

### Other Congenital Neutropenic Disorders

Cyclic neutropenia is a rare disorder characterized by regular 14- to 28-day cyclic fluctuations in the numbers of neutrophils, monocytes, eosinophils, lymphocytes, platelets, and reticulocytes. Patients develop recurrent episodes of fever, mucosal ulceration, and occasionally life-threatening infections during the period of neutropenia. Studies have shown that the administration of G-CSF at doses of 3 to 15 µg per kg per day both reduced the nadir of the neutrophil counts and shortened the duration of the nadir. G-CSF therapy was shown also to reduce the number of infections during the neutrophil nadir. [327,328,329] and [330] Chronic idiopathic neutropenia is characterized by transient maturation arrest of neutrophil precursors in the bone marrow and in neutrophil counts below 1,500 cells per $mm^3$. G-CSF has been shown to increase the neutrophil count and reduce the number of infections and need for hospitalization in these patients, [331] even at extremely low doses.[332]

## Clinical Use of Colony-Stimulating Factors: Toxicity, Dosing, and Route of Administration

### *Toxicity*

### Granulocyte Colony-Stimulating Factor

The predominant side effect associated with the administration of G-CSF is medullary bone pain, which occurs in 15% to 39% of study subjects receiving approximately 5 µg per kg per day[244] and more frequently at higher doses. Bone pain most commonly is observed either shortly after the injection or during the time of neutrophil recovery and is associated with the peak of marrow proliferative activity. It can be relieved by analgesics, such as acetaminophen or ibuprofen. Splenomegaly also has been reported to occur. Although usually asymptomatic, reports of left upper quadrant pain consistent with splenic infarction and hypersplenism have been described. Infrequent adverse effects include vasculitis, acute febrile neutrophilic dermatosis (Sweet's syndrome), osteopenia, glomerulonephritis, rashes, bone marrow fibrosis, transient leukemia cutis in a patient with chronic myelogenous leukemia, MDS or leukemia, transient inverted chromosome 5q with excess blasts, and possible anaphylactic reactions. Anti–G-CSF antibodies have not been reported. Doses of more than 100 µg per kg per day of G-CSF have been given without dose-limiting toxicity.[244]

### Granulocyte-Macrophage Colony-Stimulating Factor

At doses of 0.3 to 10 µg per kg per day, GM-CSF has been associated with fever, chills, lethargy, myalgia, bone pain, anorexia, change in weight, generalized skin eruptions, flushing, and raised erythematous lesions at the subcutaneous injection site. [244] The first dose of GM-CSF may be followed within 3 hours by a characteristic reaction involving transient flushing, tachycardia, hypotension, musculoskeletal pain, dyspnea, nausea and vomiting, and arterial oxygen desaturation and is more common after intravenous administration than after subcutaneous administration. Patients who develop this syndrome appear to be at increased risk for recurrence if GM-CSF is restarted. GM-CSF doses of more than 20 µg per kg per day are not well tolerated. The major toxicities encountered were weight gain with fluid retention, pleural and pericardial inflammation and effusions, and venous thrombosis. [244]

### Toxicity Comparisons among the Colony-Stimulating Factors

A commonly held belief is that GM-CSF may be more toxic than G-CSF. The observed GM-CSF toxicities may result from the broader range of cells stimulated, with the consequential induction of cytokines such as IL-1, TNF-a, and IL-6, [333] or of inflammatory mediators, such as the leukotrienes.[334] Randomized trials comparing G-CSF and GM-CSF in patients receiving standard-dose chemotherapy indicate relatively few toxicities for either agent. Local reactions, generalized rashes, constitutional symptoms, fever, thrombocytopenia, and anemia seem more prominent with GM-CSF.[244] However, in a randomized trial comparing the two agents as therapy for febrile neutropenia, similar efficacy for both agents and few differences in toxicity were reported. [335] Another study that prospectively compared GM-CSF and G-CSF as therapy for afebrile neutropenia also noted no differences in systemic symptoms. [336] Reports from a randomized, blinded comparison of GM-CSF and G-CSF used with standard-dose chemotherapy indicated relatively modest toxicities due to either agent, with the only major difference being a higher frequency of grade 1 or grade 2 fever with GM-CSF.[337]

### *Potential of Colony-Stimulating Factors to Induce Malignancy*

Concerns have been raised about use of GM-CSF in patients with AML. GM-CSF promotes the *in vitro* proliferation of leukemic blasts and thus might lead to *in vivo* precipitation of relapse. However, numerous clinical studies have not shown a clinically detectable adverse effect of CSFs on stimulation of myeloid leukemic cell growth. The complete response and relapse rates of patients receiving CSFs usually have been the same as or better than those in control groups. [308] However, in one trial, patients who received GM-CSF had a statistically significant increase in the incidence of residual leukemia and a decrease in disease-free survival as compared to those in the control group.[338]

Reports of MDS or acute leukemia developing during the treatment of both pediatric and adult aplastic anemia patients with CSFs have raised concerns about long-term use of these agents. Evaluating the malignant potential of CSFs in aplastic anemia is difficult, because the risk of MDS and AML can be as high as 20% in patients treated with immunosuppressive therapy (cyclosporine and antithymocyte globulin) alone. [339,340] and [341] Three cases of monosomy 7 MDS transformed from aplastic anemia after continuous therapy with G-CSF have been reported. [342] A series of 167 children with aplastic anemia included 11 who developed MDS after treatment with immunosuppression and G-CSF.[343] The incidence of MDS or leukemia has been reported to be 47% ± 17% at 7 years in 62 children who were treated with immunosuppressive therapy and G-CSF.[343] In another study, MDS developed in only 1 of 47 patients treated with G-CSF alone, as compared with 4 of 18 patients who received either antithymocyte globulin or cyclosporine. In this analysis, the administration of G-CSF for more than 1 year was the most important predictive factor in the development of MDS.[344]

Several cases of monosomy 7–related MDS and AML have been reported in patients with Kostmann's syndrome (severe congenital neutropenia) treated long-term with G-CSF.[344,345,346,347] and [348] The historical risk of malignancy in patients with Kostmann's syndrome is unknown, because before CSFs, most patients succumbed to serious infections in infancy. In a cohort of 420 patients registered with the Severe Chronic Neutropenia International Registry, 220 of whom had received G-CSF for 8 or more years, only 16 cases of AML or MDS have been reported thus far.[254] No patient with cyclic neutropenia treated with CSFs has developed a secondary malignancy.[254] All the malignancies in the Registry report were associated with mutations in the gene for the G-CSF receptor. [349] In a review of 125 patients who received G-CSF for treatment of congenital neutropenia, aplastic anemia, and MDS, 4 developed fatal MDS or AML that was related to monosomy 7. [350] Although CSFs clearly have a therapeutic effect in these severe hematologic and premalignant disorders, patients so treated must be observed carefully for the development of MDS and leukemia.

### *Dosage and Route of Administration*

### Granulocyte Colony-Stimulating Factor

The optimal dose of G-CSF has not been established in all clinical settings. Studies of primary prophylaxis after outpatient chemotherapy in adults and children have demonstrated that approximately 5 µg per kg per day of G-CSF is effective in reducing the incidence of neutropenic fever. Although some phase I investigations have suggested that higher doses of G-CSF may produce more rapid bone marrow recovery, others have not been able to document such an effect. When G-CSF is given intravenously, it is diluted with albumin to protect the G-CSF from adsorption onto plastic materials and is given as a 15- to 30-minute infusion. No reported difference in efficacy has been cited between the intravenous and subcutaneous routes of administration. [244]

Intravenous and subcutaneous administration of G-CSF doses as low as 1.5 to 2.0 µg per kg per day may shorten the duration of neutropenia after chemotherapy.[351,352] and [353] Although one of these trials suggested that the subcutaneous route was more active, both routes showed efficacy. [351] Equivalent rates of fever and comparable patterns of neutropenia have been noted with doses of 2 and 5 µg per kg per day of G-CSF, [354] and preliminary findings from a very large randomized trial evaluating fixed-unit dosing of 300 or 480 µg total per day have implied that the lower dose is as efficacious as the higher. [355] Therefore, although a G-CSF dose of 5 µg per kg per day is reasonable and safe, available data suggest that lower G-CSF doses may provide similar benefit at lower cost. [246]

In the setting of BMT, the concern that higher G-CSF doses may be required to counter extreme myelosuppression has led to randomized comparisons of doses of

10, 20, and 30 µg per kg per day. Intravenous G-CSF produced enhanced neutrophil recovery after BMT [356] and high-dose chemotherapy for acute lymphoblastic leukemia (ALL)[357] with doses of 2, 5, 10, and 20 µg per kg per day; doses exceeding 5 µg per kg per day did not appear to provide an overt additional benefit. Although sequential evaluations of G-CSF doses as high as 24 µg per kg per day have not been able to link administration of higher doses definitively with enhanced PBPC yields,[358,359] G-CSF doses higher than 10 µg per kg per day often are used when G-CSF is employed as a single agent for PBPC mobilization. [299,300] and [301,360,361] Adose-response effect for PBPC mobilization has not been established clearly.

### Granulocyte-Macrophage Colony-Stimulating Factor

The best dosing data for GM-CSF are derived from the randomized licensing trial. In it, a 2-hour intravenous infusion at a dose of 250 µg per $m^2$ per day proved effective in decreasing the time to neutrophil recovery, shortening duration of hospitalization, and lessening antibiotic use if given after autologous BMT. [93] Much of what is known is based on phase I dose-ranging evaluations of GM-CSF prophylaxis after intensive chemotherapy that have suggested activity in the range of 250 to 750 µg per $m^2$ per day in small cohorts of patients.[362,363] Phase I pediatric data implied that doses of more than 500 µg per $m^2$ per day may provide greater clinical benefits,[364] but this information is highly susceptible to selection bias. A follow-up phase II trial employing 1,000 µg per $m^2$ per day of GM-CSF after intensive chemotherapy demonstrated improvements in neutrophil counts as compared to lower doses, but showed no clinical advantages. [365] Mobilization of PBPC with GM-CSF appears to be possible with conventional doses. In one study, subcutaneous administration of GM-CSF across a dose, but range of 125 to 500 µg per $m^2$ per day indicated no evident dose-related response in the mobilization of PBPC. [366]

The ASCO guidelines recommend that G-CSF be dosed at 5 µg per kg per day and GM-CSF at 250 µg per $m^2$ per day.[247] These agents can be administered subcutaneously or intravenously, as clinically indicated. Dose escalation of CSFs is not recommended. The guidelines recommend rounding the dose to the nearest vial size in larger children and adolescents.

### Initiation and Duration of Colony-Stimulating Factor Prophylaxis

The current recommendation is that a course of G-CSF begin 24 to 48 hours after completion of chemotherapy and continue through the period of granulocyte nadir until an ANC of at least 10,000 per $mm^3$ is achieved.[244] A number of studies have evaluated alternative methods of CSF prophylaxis, striving either to initiate CSFs earlier to maximize benefit or terminate prophylaxis as quickly as possible, thereby reducing costs. [367] Starting the CSF with chemotherapy raises the concern that the CSF may induce progenitor cell cycling that actually would enhance bone marrow sensitivity to chemotherapy. [143] Evidence from studies with both G-CSF and GM-CSF suggests that this concern may be more than hypothetical. Patients receiving CSF prophylaxis simultaneously with 5-fluorouracil [368,369] plus topotecan [370] or combination chemotherapy[266] appeared to have more profound neutropenia or depressed neutrophil recovery as compared with historical controls or with patients not treated with G-CSF and chemotherapy simultaneously. More convincing has been a trial in which patients receiving daily oral etoposide were assigned randomly to receive or not receive GM-CSF concurrently with the chemotherapy; GM-CSF–treated patients had a degree of myelosuppression significantly greater than that in patients receiving only etoposide.[371] Conversely, a study of adult patients with ALL in whom G-CSF was given simultaneously with repeated courses of cell cycle–active agents (cytarabine and 6-mercaptopurine) and cyclophosphamide found that neutropenia was moderated significantly by the G-CSF, compared with the outcome in controls who received chemotherapy alone.[372] At present, avoiding concurrent use of CSFs and chemotherapy seems prudent.

Because the neutrophil nadir usually is delayed by 5 to 7 days after administration of chemotherapy, consideration has been given also to delaying the initiation of prophylaxis with CSFs until the expected onset of severe neutropenia. Eighteen children were randomly assigned to early initiation of G-CSF therapy (24 hours after chemotherapy) or delayed initiation (5 days after chemotherapy). No differences were noted between the two groups in terms of infection, hospital days, and intravenous antibiotic use.[373] In another study, 72 courses of chemotherapy were given in a sequential cohort study. G-CSF was administered either 24, 48, 72, or 96 hours after chemotherapy. Patients who received G-CSF early (24 and 48 hours after chemotherapy) had a shorter duration of leukopenia as compared to that in patients who received G-CSF later (72 or 96 hours). However, no difference was seen in the duration of ANC below 500 cells per cubic or thrombocytopenia. [244] In a study of patients undergoing autologous PBPC collections, 30 received G-CSF immediately after the end of chemotherapy and 35 received the first G-CSF dose 3 to 4 days after the completion of chemotherapy. The study reported no statistically significant difference between the two groups in the median numbers of $CD34^+$ cells collected or the rate of failure to collect an adequate number of stem cells. [374]

The ASCO guidelines recommend starting G-CSF or GM-CSF between 24 and 72 hours after chemotherapy to provide optimal recovery. [247] Continuing the CSF until the occurrence of an ANC of 10,000 cells per $mm^3$ after the neutrophil nadir, as specified in the G-CSF package insert, is known to be safe and effective. However, a shorter duration of administration sufficient to achieve and sustain clinically adequate neutrophil recovery is a reasonable alternative, considering issues of patient convenience and cost.

## HEMORRHAGE

The body's chief defense against hemorrhage from large blood vessels is rapid deposition of an insoluble fibrin clot. This results from a complex interaction of circulating coagulant and anticoagulant proteins finely tuned to maintain hemostasis. If the system becomes unbalanced by infection, neoplasia, or complications of its treatment, hemorrhage or thrombosis may result.

### Disseminated Intravascular Coagulation

DIC is characterized by increased amounts of thrombin and plasmin in the circulation. This increase results in consumption of platelets, coagulation factors and inhibitors, hemolytic anemia, and secondary hyperfibrinolysis. Ultimately, DIC results in microthrombi formation and acute generalized bleeding. [375,376]

Screening laboratory tests indicative of DIC include a prolongation of the prothrombin time (PT) and the activated partial thromboplastin time (APTT) due to consumption and proteolysis of fibrinogen and other coagulation factors. Some patients may demonstrate shorter than normal PT and APTT values owing to circulating activated clotting factors. [375,377,378] Thrombocytopenia is a constant finding in DIC, resulting from shortened platelet survival. In addition, DIC may cause platelet dysfunction.[379] However, thrombocytopenia may be related also to the underlying disorder, particularly in cancer patients. Schistocytes often are seen on the peripheral blood film. Elevated fibrin degradation products (FDP) or fibrin split products almost universally are seen in DIC, but the levels may be influenced by the balance of procoagulant activity, fibrinolytic response and activity of the renal and macrophage mediated clearance mechanisms. D-Dimer is more specific but is less sensitive than FDP measurements.[375,377,378] Specific factor assays, antithrombin levels, and fibrinopeptide A measurements usually are not required in clinical practice unless administration of a specific replacement product is contemplated.

In children with cancer, DIC is seen predominantly with gram-negative septicemia. Laboratory evidence of DIC has been reported in 3% to 13% of patients with newly diagnosed pediatric acute leukemia. It is more common in AML, particularly the promyelocytic (M3) and monomyeloblastic (M5) subtypes, and in children with ALL and hyperleukocytosis, particularly with a T-cell phenotype. [380,381] DIC has been reported also in children with neuroblastoma and other solid malignancies, usually in the setting of diffuse metastatic disease or extensive primary tumors.[382,383]

Suppression or attenuation of the underlying cause of DIC remains the most crucial aspect of treatment. Excellent critical care, including meticulous correction of hypovolemia, hypoxia, and acidosis, is required. Blood products to replace the consumed platelets, coagulation factors, and inhibitors are indicated for intractable bleeding or in preparation for invasive procedures. [375,378] Replacement of factors with cryoprecipitate, FFP, or platelet concentrates does not appear to “fuel the fire” significantly by causing thrombosis in active DIC. [384,385]

Replacement therapy, if given, should be as specific as possible and usually is provided by FFP at a dose of 10 to 15 mL per kg body weight. Large volumes, up to 6 units per 24 hours, often are required in adults. [378] If this dosage does not result in cessation of bleeding or in a fibrinogen level of more than 0.5 g per L, cryoprecipitate, 10 units per 2 to 3 units of FFP, can be added. [375] Platelet transfusion should be considered for values of less than 20,000 per $mm^3$ or for major bleeding if the platelet count is less than 50,000 per $mm^3$.[375,378]

Heparin therapy for DIC is less widely recommended than previously, reflecting the absence of objective data demonstrating its benefit. Some physicians consider this agent useful to prevent the formation of thrombi and subsequent organ dysfunction caused by ischemia. Heparin should be considered only in DIC with overt thromboembolism or with extensive deposition of fibrin, such as in purpura fulminans, [378,386] chronic DIC, thrombosis often associated with adult cancers (Trousseau's

syndrome),[387] and perhaps in the coagulopathy associated with APML.[388]

APML (M3) frequently is associated with a distinct form of DIC. In such cases, a severe hyperfibrinolytic state and an activated coagulation system lead clinically to bleeding, but disseminated thrombi often are found at autopsy.[378,389] Induction treatment that includes all-*trans*-retinoic acid, which induces differentiation of myelocytes into neutrophils, rapidly normalizes fibrinogen concentration leading to amelioration of the bleeding diathesis, instead of worsening it as is seen with chemotherapy alone.[390] However, the thrombotic tendency due to persistent procoagulant activity seen in patients with APML who develop hyperleukocytosis may persist for several months.[391] Treatment with arsenic trioxide, a promising therapy for patients with relapsed or refractory APML, also appears to decrease FDP and D-dimer levels.[390] A low-dose constant infusion of heparin during induction chemotherapy formerly was believed to be of value, but today it usually is reserved for marked or persistent elevation of FDP or clinical thrombosis.[388]

Thus, for DIC with overt thromboembolism or extensive deposition of fibrin (as occurs in purpura fulminans), unfractionated heparin may be given at a low dose of 5 to 8 units per kg per hour as a continuous infusion.[378,392] Low-molecular-weight heparin also has been used in this setting, with equal efficacy and a decrease in bleeding.[393,394]

Antithrombin levels may be low in DIC, and infusion of antithrombin concentrates with or without heparin may be beneficial. The outcome of an ongoing randomized multicenter trial to administer high doses to patients with sepsis will help to determine the role of this agent in the treatment of DIC.[376,378,392] Similar trials of activated protein C concentrates are under way. Numerous other novel therapies for DIC, including the protease inhibitors gabexate and aprotinin, also are under evaluation.[376] Clinical use of these agents should be limited to investigational protocols at present.

## Specific Clinical Problems in Hemostasis

### *Liver Disease*

Patients with impaired hepatocellular function have a bleeding tendency, primarily originating from decreased synthesis of multiple blood coagulation factors in the liver. Children with cancer may develop severe hepatic dysfunction, hepatitis, or cirrhosis as a consequence of transfusion-related infection, hepatotoxicity of chemotherapy, biliary obstruction, or sepsis. A prolonged PT in this setting can be due to multiple causes, and the need for clotting factor replacement must be ascertained by measurement of coagulation factor levels. Although recommended management of a prolonged PT consists of FFP as treatment of hemorrhage or before invasive procedures, few data addressing efficacy are available.

### *Vitamin K Deficiency*

Vitamin K deficiency occurs infrequently in children with cancer and usually is due to diminished intake, decreased intestinal absorption, or suppression of bowel flora by broad-spectrum antibiotics (resulting in reduced synthesis of endogenous vitamin K). Without vitamin K to promote g-carboxylation of the glutamic acid residues of factors II (prothrombin), VII, IX, and X, these factors are unable to bind calcium and are inactive in the clotting cascade. Factor VII has the shortest half-life, so the PT becomes prolonged first. Vitamin K is required also for the function of the natural circulating anticoagulants protein C and protein S. Fibrinogen concentration and platelet counts usually are normal in vitamin K deficiency.

Therapy consists of oral or parenteral vitamin K (1 to 5 mg) daily for a minimum of 3 to 5 days. Oral vitamin K may be absorbed erratically, whereas intravenous therapy may be associated rarely with hypotension. Patients who fail to respond to vitamin K or who urgently require invasive procedures may be treated with FFP. Given the short half-life of factor VII (approximately 6 hours), aggressive support with FFP (10 to 15 mL per kg every 6 to 8 hours) often is necessary to produce a sustained reduction of the PT. Rarely does the PT shorten to less than 14 seconds.[377]

### *Massive Transfusion Syndrome*

Massive transfusion syndrome results from transfusion of large amounts of stored red blood cells over a short interval (usually hours) without concomitant transfusion of FFP or platelets. Initially, it was thought to be due to a “wash-out” of plasma clotting proteins and platelets.[377] More recently, the problematic diffuse microvascular bleeding in this syndrome was demonstrated to be due to a low platelet count.[395] Transfusion of a red cell volume equal to 1.5 times the blood volume or more over a period of hours may place a patient at risk. Treatment consists of laboratory assessment of the platelet count, PT, APTT, and FDPs with replacement by transfusion of the deficient components, usually platelets. In the setting of massive transfusion and bleeding, the platelet count should, if possible, be maintained at more than 50,000 per $mm^3$, owing to the functional platelet abnormalities that may occur also in this situation.[395,396]

### *Uremia*

Uremia is a paradigm of the entire coagulation system, predisposing to both bleeding and thrombosis. Bleeding, usually mucosal or with invasive procedures, is related to the degree of uremia.[397,398] Mechanisms that promote bleeding include quantitative and qualitative platelet defects resulting from contact with small and intermediate-weight dialyzable factors, from vitamin K deficiency, and from a circulating heparin-like anticoagulant.[397,398 and 399] Although uremic bleeding is partially correctable by hemodialysis, maintaining the hemoglobin at more than 10 g per dL may promote the formation of the primary platelet plug, the nidus of the clot.[400] The use of 1-deamino-8-D-argenine vasopressin (DDAVP) in standard doses may correct the bleeding time and allow invasive procedures if dialysis and adjustment of the hemoglobin do not.[401] Nephrotic syndrome and dialysis predispose to thrombosis through a variety of mechanisms, including increased levels of coagulation factors and decreased levels of antithrombin.[397,399]

### *Therapy for Hemorrhage*

Children with cancer and altered laboratory tests of hemostasis but no hemorrhage do not require treatment. FFP is used to treat coagulation disorders, but generally it should not be used as a volume expander or as prophylaxis of coagulation abnormalities.[19,402] FFP should be ABO-compatible with the patient's red blood cells. It requires 30 to 60 minutes to thaw in a manner that preserves the function of its coagulation factors.[19] The usual dose of FFP is 10 to 15 mL per kg administered over 1 to 2 hours as volume considerations allow. It may be repeated every 12 to 24 hours as needed to stop hemorrhage. Each unit of FFP contains on average 1 unit per mL of prothrombin and factors V, VII, VIII, IX, and X in a volume of 180 to 300 mL.[19,20] It can bestored frozen for 1 to 7 years, depending on conditions. Units can be held in quarantine until the donor returns for repeat testing after an interval longer than the window period for known viruses and the units can be shown to be seronegative. This approach to ensure the safety of transfused plasma has been used in the United States since September 1998.[230]

Treatment of plasma with a solvent-detergent (SD) process to inactivate lipid envelope viruses is another approach to producing plasma with a decreased infection risk. The production of SD plasma is accomplished by pooling 2,500 individual plasma donations, treating them with solvent and detergent, and repackaging the product in standardized 200-mL units. The contents are unchanged from single-donor units except that the procoagulant activity is reduced by 15% to 30%, proteins C and S by 20% to 40%, and large multimers of von Willebrand factor by 50% or more.[230,402] A unit of SD plasma costs two to five times as much as FFP.[403]

The large plasma pools necessary to render SD processing commercially feasible raise concern about transmission of nonenveloped viruses. The product does not transmit HIV-1 or HIV-2, HBV or HCV, HTLV-1 or HTLV-2, or HGV.[404] SD treatment is not effective against HAV,[230] parvovirus B19,[230] and the newly described liver disease–related TT virus.[405,406] Convenience and lack of knowledge that SD plasma is blood-derived also may lead to inappropriate use, just as it has with albumin.[230,402,403]

## THROMBOSIS

Thrombosis recently emerged as a significant problem in children with cancer. The most common sites of deep venous occlusion are the iliofemoral system in the legs and pelvis and the subclavian veins and superior vena cava in the chest. Arterial thrombosis is encountered as well.

### Predisposing Factors

The pathophysiology of thrombosis is complex, involving a number of acquired and underlying genetic alterations. First, as addressed in the discussion of DIC, an ill-defined hypercoagulable state exists in cancer patients of all ages. [407] Notably in children, this occurs with leukemia (especially with hyperleukocytosis or AMPL) or disseminated solid tumors.[380,381,382 and 383] Second, as discussed later, obstruction of flow in the upper venous system due to implanted central venous catheters predisposes to thrombosis.[408,409] Certain medications, particularly L-asparaginase, may further promote a prothombotic state. Finally, immobilization or obstruction of venous return due to massive adenopathy, hepatosplenomegaly, or bulky tumor may predispose children with cancer to development of a clinically significant clot.

Underlying genetic disorders predisposing to thrombosis may provide an added risk. Although inherited deficiencies of antithrombin, protein C, and protein S are uncommon, the factor V Leiden (factor V G1691A) mutation, prothrombin G20210A mutation, the TT677 genotype of methylenetetrahydrofolate reductase (MTHFR), and elevated lipoprotein(a) concentrations cumulatively affect more than 10% of the general pediatric population with stroke. [410] That inherited thrombophilias may contribute to thrombosis in certain populations was suggested by the European cooperative group finding that 67% of pediatric patients with ALL and thrombosis—but only 21% of ALL patients overall—had a genetic risk factor. [411] Further, no patient with other forms of pediatric leukemia, lymphoma, or solid tumors and thrombosis had a genetic risk factor.[411] This group also recently reported that 22 of 289 children (11%) with ALL treated on the Berlin-Frankfurt-Münster 90 and 95 protocols developed deep venous thrombosis. Some 27 of 58 patients (46.5%) with at least one pro-thrombotic risk factor developed thrombosis as compared to 5 of 231 (2%) of patients without any risk factors.[412] Although arterial thrombosis may occur as well, also secondary to indwelling catheters, these events are not nearly as common as those involving large veins. [413]

Diagnosis of venous thrombosis in the lower extremities is best made by compression duplex Doppler ultrasonographic examination. Although line studies involving instillation of contrast into the implanted device can disclose obstruction at the tip or interruption of the catheter, contrast venography is required for definition of thrombi in the upper venous system. This test involves injection of a small amount of contrast material into an antecubital vein followed immediately by fluoroscopy or plain film images of the chest.

#### *Central Lines*

Early in their treatment, children with cancer frequently have a central venous catheter placed for ease of infusion of fluids, chemotherapy, and blood products. Small thrombi occluding the tip of the catheter are thought to be prevented by regular heparin flushes, although this preventative strategy is increasingly questioned. [414] Larger thrombi may occlude the lumen and lead to inability to withdraw blood. Catheters also alter the flow of blood and cause endothelial injury that predisposes to thrombosis by their very presence.

The most common form of deep venous thrombosis in children with cancer is stenosis or occlusion of veins in the upper venous system, including the superior vena cava and subclavian, bracheocephalic, and jugular veins. Such thrombi may occur shortly after catheter insertion and result acutely in superior vena cava syndrome (e.g., facial swelling and cyanosis, distended neck veins, swelling of the upper arm). [415] More commonly, thrombi are detected by difficulty in accessing the catheter, a prominent pattern of distended veins on the chest wall and neck, and gradual swelling of the extremity distal to the obstruction. [408,409] Pulmonary embolism, with fatal sequelae, has been reported.[408,409] A recent prospective study of the prevalence of upper venous system thrombosis in children with cancer and had implanted venous catheters for 6 months or more showed that 50% of patients had a radiographically identified major thrombus involving the upper venous system with prominent collateral vessels. Most of the patients were asymptomatic.[416] Similarly, the European group noted a “clear-cut positive correlation” between thrombosis in children with ALL and the use of central lines. [412]

Treatment with either low-dose low-molecular-weight heparin[417] or mini-dose warfarin (1 mg per day in adults)[418,419] starting after catheter insertion has been demonstrated to decrease the incidence of thrombus development[418,419 and 420] or recurrence[420] in patients with cancer.

#### *L-Asparaginase*

Intensive therapy with L-asparaginase is used in most ALL treatment protocols in childhood. For more than two decades, combined hemorrhagic and thrombotic lesions involving the central nervous system accompanied by clinical stroke have been reported in 2% to 3% of children with ALL and receiving L-asparaginase during induction therapy.[412,421] Large thrombi in other veins, and more recently a high incidence of catheter-related upper venous system thrombosis, also have been reported in patients receiving repeated doses of L-asparaginase.[409,412]

This agent decreases antithrombin and plasminogen levels predisposing to thrombosis. [421,422,423 and 424] Protein C and protein S deficiency do not appear to play a role.[425] This agent has been associated also with significant lipid abnormalities and pancreatitis that may themselves have a role in the development of thrombosis. [426] Trials are under way to determine whether infusion of antithrombin concentrates during leukemia induction therapy reduces the incidence of this complication. FFP infusions, although often employed in the past, do not appear to be of value.

#### *Treatment*

No specific management recommendations exist for the treatment of thrombosis in children with cancer, and a full review of all options is beyond the scope of this chapter. A number of recent comprehensive reviews address various aspects of the problem.[427,428,429,430,431,432 and 433] Theapproach to management includes a decision about systemic thrombolysis with tissue plasminogen activator or streptokinase if the clot is large and recently formed versus instillation of urokinase or lower doses of tissue plasminogen activator into the catheter if only mild problems with aspiration from or infusion into the catheter are noted. Removal of the catheter, if present, is indicated for all but promptly resolving thrombi. The need to continue to treat the malignancy may require the cautious continuation of L-asparaginase along with anticoagulation. Further, the need for intrathecal administration of chemotherapy and anticipated thrombocytopenia with even moderate-dose chemotherapy may complicate the planning of anticoagulation.

Patients with acute symptomatic thrombi should receive anticoagulation with either low-molecular-weight heparin at therapeutic doses or conventional unfractionated heparin followed by warfarin. If instituted rapidly, this treatment may prevent the sequelae of thrombosis, such as post-phlebitic syndrome. [427] Treatment should be continued until the clot resolves or stabilizes and for an additional period (6 weeks to 6 months) to allow vessel healing. The need for therapy is less clear in patients who are found coincidentally or on the basis of minor symptoms to have obstructed catheters many months after catheter placement.

## CONCLUSION

The expanded use of hematopoietic growth factors, understanding of the potential complications of central line use, and safer blood products have improved the hematologic supportive care available to pediatric oncologists. Further investigations, including cost-benefit analyses with assessment of quality of life parameters, will be necessary to refine further the optimum care of children with cancer.

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# INFECTIOUS COMPLICATIONS IN PEDIATRIC CANCER PATIENTS

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Chapter References

## NATURE OF THE IMMUNOCOMPROMISED HOST

Infections cause significant morbidity and mortality in children with cancer. Cancer itself, independent of its therapy, puts children at risk for serious infection. For example, leukemia can disturb normal immune function. Solid tumors can cause anatomic obstruction with subsequent infectious complications. Tumors can also lead to functional impairments (e.g., poorly coordinated swallowing in patients with brainstem tumors) that contributes to significant infectious risks.

The therapy for cancer involves three primary modalities: chemotherapy, surgery, and radiation therapy, each of which contributes to the risk of serious infections. A large proportion of the drugs used for cancer therapy are myelosuppressive and toxic to the mucosal epithelium, putting patients at risk for serious bacterial and fungal infections. Radiation therapy can cause local tissue breakdown and provide potential sites for focal infections. Surgery has inherent associated infectious risks made more substantial by the underlying immunosuppressed status of the child. In addition, stem cell transplantation carries with it a degree of immune suppression, both acute and chronic, that puts children at significant risk. The effects of host colonization, disruption of natural skin and mucosal barriers, presence of foreign bodies, and overall nutritional status of the patient further influence the risk for infection ( Fig. 41-1).

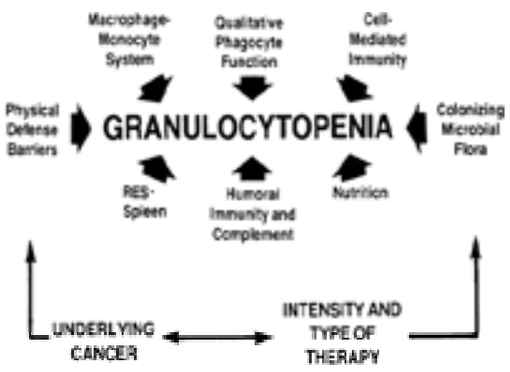



**FIGURE 41-1.** Interactions of the defense matrix that delineate the compromised host. RES, reticuloendothelial system.

It is important to recognize that there are variations among patients in the degree to which they are immunocompromised, the type of impairment, and, consequently, the types of infections to which they are susceptible. An understanding of the nature of the immune defects, the clinical presentation of common infectious syndromes, the likely pathogens, and the appropriate management of these situations is critically important for individuals providing medical care for these patients.

## INTEGUMENTARY BARRIERS, ALTERED MICROBIAL COLONIZATION, AND ENHANCED SUSCEPTIBILITY TO INFECTION

The skin and mucosal surfaces constitute the primary host defense against invasion by endogenous and acquired microorganisms. A complex arrangement of specialized cells of the skin and the respiratory, gastrointestinal (GI), and genitourinary mucosa protect the body from invading pathogens. For example, ciliated and mucus-producing cells of the respiratory tract, acid- and enzyme-producing cells of the GI tract, and bactericidal fatty acids and secretory immunoglobulins displayed by epidermal cells are specific adaptations that enhance defense against exogenous organisms.

Any factor that bypasses or disrupts the integumentary barrier can enhance susceptibility to infection. The integrity of this barrier may be disrupted by local tumor invasion or as a result of surgery, irradiation, or cytotoxic chemotherapy. Mucositis, for example, is an important feature of therapies that are toxic to the cells of the GI mucosa, rendering the patient vulnerable to infection by bacteria that reside in the GI tract. Many procedures, including fingersticks, venipunctures, bone marrow aspirations, and insertion of venous access devices, can also disrupt the integument and provide a nidus for colonization and the eventual dissemination of pathogens.

Mucosal and epithelial cells also contain specific and nonspecific receptors for the attachment or adherence of bacteria. The microbial ligands for these receptors are called adhesins. The attachment of a microbial pathogen to an epithelial surface receptor by means of adhesins is often the first step in the initiation of an infectious process.[1,2] In healthy persons, integumentary and mucosal attachment sites are populated with relatively innocuous “normal” flora, consisting predominantly of aerobic gram-positive and a variety of anaerobic organisms.[1,3] Within 24 hours of hospitalization, seriously ill patients undergo a change in their indigenous microflora toward one of aerobic Gram-negative organisms.[4,5] Why this change occurs is unclear, although underlying disease and exposure to antibiotics can promote changes in bacterial adherence and colonization.[6]

The organisms colonizing the patient are integrally related to the pattern of infections that ultimately occurs in the cancer patient. More than 80% of the microbiologically documented infections that occur in patients with acute myelogenous leukemia (AML) are caused by organisms that are part of the endogenous microflora, usually at sites at or near the source of infection. Approximately half of the responsible pathogens are acquired by the patient after the initial admission to the hospital.

### Cellular Immune Dysfunction

The effector cells of the immune response include polymorphonuclear leukocytes (PMNs), T lymphocytes, B lymphocytes, natural killer cells, peripheral blood monocytes, and fixed-tissue macrophages (including the cells of the spleen and reticuloendothelial system). The cells that are more severely affected by disease or therapy-mediated immune dysfunction are the PMNs, monocytes, and lymphocytes. The cells of the reticuloendothelial system are relatively less sensitive to the toxic effects of antineoplastic therapy and provide at least a rudimentary framework for the maintenance of nonclonal cellular immunity even in the face of profound cellular immunosuppression. Quantitative or qualitative abnormalities of the cellular aspects of the immune response predispose the patient to infections with a broad array of pathogens (Table 41-1).

| |
|---|
| **Gram-positive bacteria** |
| Staphylococci (coagulase negative, *Staphylococcus aureus*) |
| Streptococci (α-hemolytic) |
| Enterococci |
| Corynebacteria |
| *Listeria* sp. |
| *Clostridium difficile* |
| **Gram-negative bacteria** |
| Enterobacteriaceae (*Escherichia coli*, *Klebsiella*, *Enterobacter*, *Serratia*) |
| *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* (and similar oxidase-positive multiresistant gram-negative organisms) |
| Anaerobes |
| **Fungi** |
| *Candida* sp. |
| *Aspergillus* sp. |
| Zygomycetes |
| Cryptococci |
| **Other** |
| *Pneumocystis carinii* |
| *Toxoplasma gondii* |
| *Strongyloides stercoralis* |
| *Cryptosporidium* |
| **Viruses** |
| Herpes simplex virus |
| Varicella-zoster virus |
| Cytomegalovirus |
| Epstein-Barr virus |
| Respiratory syncytial virus |
| Adenovirus |
| Influenza virus |
| Parainfluenza virus |

**TABLE 41-1. PREDOMINANT PATHOGENS IN PEDIATRIC CANCER PATIENTS**

### Polymorphonuclear Leukocytes

A critical determinant of susceptibility to most bacterial and fungal pathogens in patients receiving cytotoxic chemotherapy is the number of circulating neutrophils.

The more profound and protracted the granulocytopenia, the greater the likelihood of a serious infection. [7] Granulocytopenia may be secondary to the disease itself (e.g., acute leukemia or aplastic anemia) but is more commonly a consequence of cytotoxic chemotherapy or radiotherapy. The absolute level of granulocytopenia is a particularly important risk for infection; most bacteremias and bacterial pneumonias occur when the absolute neutrophil count is less than 100 cells per $mm^3$.

The second crucial determinant of outcome is the rate at which granulocyte numbers return to a "protective" level after the onset of fever. Neutropenia that persists, usually for more than 1 week, is associated with increasing risk for recurrent or new infections, both bacterial and fungal. On the other hand, those in whom no infection has been documented, who defervesce rapidly on empiric microbial therapy, and who resolve granulocytopenia within 1 week of the febrile episode are more likely to pursue an uncomplicated course. Even in the setting of a documented infection, rapid return of the granulocyte count (i.e., within 7–10 days) is usually predictive of a good outcome.

Finally, the rate at which the granulocyte count declines also contributes to the susceptibility to infection. A patient with aplastic anemia in whom the granulocyte count has decreased slowly over a period of weeks may be at lesser risk of a serious bacterial infection than is the patient with a similar degree of granulocytopenia induced within days by antecedent chemotherapy. However, the prolonged neutropenia associated with aplastic states predisposes to serious, often fatal fungal infections.

Qualitative abnormalities of neutrophil function may occur as a consequence of the underlying malignancy (especially acute leukemias) or secondary to antineoplastic therapy. For example, the neutrophils from patients with leukemia or lymphoma exhibit suboptimal chemoattractant responsiveness, bactericidal activity, and superoxide production.[8,9] Antineoplastic chemotherapy and radiotherapy also cause qualitative abnormalities of neutrophil function. [10,11] Deficiencies of neutrophil function iatrogenically induced by the administration of various medications (e.g., opiates, corticosteroids, antibiotics) may have a detrimental effect. [12] Patients with quantitative or qualitative defects of their PMNs are subject primarily to infections with bacteria and fungi. Gram-positive and gram-negative bacteria and invasive fungi (especially *Candida* and *Aspergillus*) are the most common pathogens.

### Lymphocytes

Disease- or treatment-induced abnormalities of lymphocytes can affect both the humoral (primarily B-cell–mediated) and the cellular (primarily T-cell–mediated) arm of the immune response. Certain malignancies, such as chronic lymphocytic leukemia or multiple myeloma, result in a significant alteration of the humoral limb of the immune response—that is, the ability to generate antigen-specific neutralizing antibodies against pathogens. [13,14] Patients with defects in humoral immunity are particularly susceptible to infections by encapsulated bacteria, especially *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis*. Patients with Hodgkin's disease or non-Hodgkin's lymphoma have an impaired cellular immune response. Corticosteroids and radiotherapy can also contribute to lymphocyte dysfunction.[12,13,14,15] and [16] Patients receiving T-cell–depleted bone marrow transplants are particularly susceptible to viral pathogens, especially cytomegalovirus (CMV), although they may have a lower incidence of graft-versus-host disease. Furthermore, pathogens such as CMV that infect patients with altered cellular immunity can further suppress the host's defenses.[17,18] Patients with deficiencies of cellular immunity are prone to fungal, viral, and intracellularly replicating bacterial pathogens (e.g., *Listeria monocytogenes*, *Salmonella* sp.).

Depletion of helper T cells ($CD4^+$) is clearly induced by cytotoxic chemotherapy. Mackall and colleagues [19] have shown that although neutrophil, monocyte, and platelet numbers consistently recover to greater than 50% of pretreatment values after sequential cycles of therapy, lymphocyte numbers do not recover promptly, and lymphopenia may persist for many months after the completion of a chemotherapy regimen. The capacity for $CD4^+$ T-cell regeneration after chemotherapy seems to diminish with age, so that younger children have significantly greater recovery of T cells 6 months after chemotherapy than do young adults who have persistent, severe T-cell depletion. It appears that the thymus-dependent regeneration of T cells plays an important role in younger children, whereas older children and young adults have deficiencies in this pathway, perhaps as a result of the normal thymic involution that occurs with age. Prolonged T-cell depletion probably contributes to the development of opportunistic infections such as herpes zoster or *Pneumocystis carinii* pneumonia (PCP) during the months after chemotherapy.

### Spleen and Reticuloendothelial System

The spleen and fixed tissue cells of the reticuloendothelial system act as a mechanical filter and as an immune effector organ. The spleen is the principal organ involved in the production of antibodies to polysaccharide antigens, and it also filters out damaged cells and opsonin-coated organisms from the circulation. [20] Splenectomized patients are deficient in antibody production when challenged with particulate antigens, have decreased levels of immunoglobulin M (IgM) and properdin (a component of the alternate complement pathway), and are deficient in the phagocytosis-promoting peptide tuftsin. [21] Splenectomized patients are at increased risk for fulminant and rapidly fatal septicemia caused by encapsulated bacterial pathogens such as *S. pneumoniae, H. influenzae,* and *N. meningitidis*.[22]

### Other Factors Contributing to Immunocompromised States

Additional factors can exacerbate the immunocompromised state in a child with cancer. Alterations in central nervous system function or decreased levels of awareness, obstruction of a hollow viscus, and the depressed nutritive states common with malignancy can enhance susceptibility to infectious complications.

Obstruction of the biliary tree, GI or genitourinary tract, or respiratory passages by a primary or metastatic tumor mass can promote an infection by the organisms colonizing the site of obstruction. Similarly, diminution or obliteration of the gag reflex secondary to local neural infiltration or a decreased level of cognition markedly increases the risk of aspiration pneumonia. Aspirated pharyngeal organisms (most commonly gram-negative aerobes) can colonize, invade, and disseminate from a pulmonary source. The risk of aspiration pneumonia and subsequent disseminated infection is heightened by decreased mucosal clearance mechanisms and damage mediated by antineoplastic therapy.

The compromising effect of a malnourished state on immune function is well documented. [23] Although the precise effects of nutritional deficiencies in cancer patients have been difficult to elucidate, it is clear that nutritional deficiencies affect B and T lymphocytes, PMNs, mononuclear phagocytes, and complement system function.

## CARE OF THE FEBRILE CANCER PATIENT

### Definitions of Fever and Neutropenia

Fever (i.e., a temperature greater than 38 $^oC$) is common among children with cancer.[24] The production of fever in humans is a process mediated by the actions of several proinflammatory cytokines, primarily interleukin-1 (IL-1), tumor necrosis factor (TNF), and IL-6. These cytokines are produced by myriad cell types, although the monocyte-macrophage is their major source. They share a number of proinflammatory properties that inhibit bacterial replication, including the induction of fever, hepatic synthesis of acute phase reactants, activation of T and B cells, and metabolic changes such as mobilization of amino acids, decreases in serum iron and zinc, and increase in serum copper. By inducing the synthesis of other mediators, IL-1 and TNF also stimulate neutrophil, lymphocyte, and monocyte migration and activate mature neutrophil functions such as chemotaxis, phagocytosis, and killing of bacteria. IL-1 and TNF also appear to mediate the development of septic shock in humans. The overall effect of these activities is a unified host response against an infectious insult, with fever being an important by-product. [25]

Although fever in cancer patients is frequently caused by infection, noninfectious causes must also be considered. Pyrogenic medications (particularly cytotoxic agents such as bleomycin and cytosine arabinoside), blood products, allergic reactions, and the malignant process itself are potential sources of a febrile response. Nonetheless, in a granulocytopenic patient, fever may be the first and only sign of infection. [24,26] Other clinical signs and symptoms frequently indicative of an infectious process (i.e., pain, erythema, swelling) may be blunted or lacking. Alternatively, localized pain and signs of inflammation may occur in the absence of fever in a neutropenic patient. The physician must pay careful attention to these indicators of possible underlying infection in this situation and strongly consider antibiotic therapy even if there is not a febrile response.

Although the definition of granulocytopenia is somewhat arbitrary, most oncologists consider patients with an absolute granulocyte count (consisting of PMNs plus band forms) of 500 cells per $mm^3$ or less to be neutropenic. From a practical standpoint, patients whose absolute count is between 500 and 1,000 cells per $mm^3$ but falling because of antineoplastic therapy should be considered neutropenic.

### Pediatric versus Adult Patients

Pediatric cancer patients are different from their adult counterparts in multiple ways. These include the spectrum of oncologic diagnoses, the intensity of

chemotherapeutic regimens (with a larger percentage of children with cancer treated very intensely with the goal of curative therapy), and the incidence and severity of comorbid medical conditions preceding the diagnosis of cancer. In addition, the differences in prophylactic antimicrobial use, the percentage of patients with indwelling central venous catheters, the community exposures to infectious pathogens, and the maturation of the immune system may also be different based on age.

These differences between adult and pediatric patients affect the frequency and nature of episodes of fever and neutropenia. A review of results from four studies performed by the European Organization for Research on the Treatment of Cancer highlighted some of these differences. They reported that the sites of infection and spectrum of infecting organisms are different in children and adults. [27] Children more often do not have a clinically apparent site of infection and consequently have a higher rate of fever without a source. When a defined site is present, children were more likely than their adult counterparts to have upper respiratory tract findings. The overall incidence of bacteremia is similar; however, the rate of death during fever and neutropenia was 1% in children compared to 4% in adults.

**Evaluation of the Patient with Fever and Neutropenia**

The initial assessment of patients with fever and neutropenia has not changed significantly over the past three decades. A careful history and meticulous physical examination are extremely important. Neutropenic hosts have a decreased ability to manifest an inflammatory response, and thus even subtle signs and symptoms should be considered significant. Attention should be paid to areas at special risk in patients receiving cytotoxic therapy, such as the oropharynx, respiratory tract, perirectal area, central venous line sites, and any site of recent invasive procedures.

Blood cultures should be obtained from all lumens of central venous lines, when present, as well as peripherally. Other cultures should be obtained based on clinical suspicion (stool, urine, cerebrospinal fluid, central line site, or wound). [27] Routine serum chemistries should be obtained to ensure appropriate supportive care and safe administration of antimicrobials and other needed medications.

A chest radiograph should be considered in all patients. Although the yield of routine chest radiographs in asymptomatic neutropenic patients is small, the study should be performed in all patients with projected prolonged neutropenia because it provides an important baseline for comparison with later films, which may present only subtle indications of a pneumonic process. Other radiological studies may be helpful based on specific symptoms or physical examination findings.

After this evaluation, broad-spectrum antibiotics should be started promptly in all febrile neutropenic patients.

**Evaluation of the Afebrile Neutropenic Patient with Localizing Signs**

Granulocytes play an important role in the development of inflammatory responses to infection, and consequently, during neutropenia, inflammation may be markedly attenuated despite ongoing infection. Fever itself can be absent in some cases. The presence of infection may be detected only by attention to seemingly minor complaints from the patient or by subtle localized physical findings. It is critical that the physician takes these complaints or findings seriously and pursues them vigorously. The absence of fever in cancer patients with localizing signs does not mean that an underlying infection is controlled or insignificant. Abdominal pain, for example, may signify an evolving intra-abdominal infection (e.g., typhlitis), whereas erythema and tenderness along a subcutaneous catheter tunnel track usually indicate the presence of a deep soft tissue infection, even if the patient is afebrile. In these situations, it is most prudent to obtain cultures of blood and any other pertinent sites and then immediately to begin antibiotics directed against probable pathogens. Any delay in antibiotic therapy while awaiting the results of cultures allows for unchecked progression of infection in the neutropenic host.

**Risk Assessment in Cancer Patients with Fever and Neutropenia**

Traditionally, the empiric therapy for oncology patients with fever and neutropenia has involved admission to the hospital and administration of broad-spectrum intravenous (i.v.) antibiotics. [28] More recently it has become clear that not all patients with fever and neutropenia are at equal risk for significant morbidity or mortality from infection. [29,30,31 and 32] (The identification of a low-risk subset may allow for modifications of therapy in this group, with a goal of less therapy-related toxicity, an improved “quality of life” and decreased cost.)

A retrospective study performed by Talcott and colleagues [29] evaluated risk factors for serious medical complications and death during episodes of fever and neutropenia in adult oncology patients. A “high-risk” group was defined as those patients who were inpatients at the time of diagnosis with fever and neutropenia, or those presenting as outpatients with either concurrent comorbidity or uncontrolled cancer. The “low risk” group was, by exclusion, those patients presenting with fever and neutropenia as outpatients without comorbidity or progressive cancer. Importantly, the information required to stratify a patient as either high or low risk was available to the clinician at the time of the patient's presentation with fever and neutropenia. The medical course in the two groups was found to be significantly different. The rates of serious complications ranged from 31% to 55% in the high-risk group, compared to 2% in the low-risk group. Similarly, rates of death ranged from 14% to 23% in the high-risk group. No patients died in the low-risk group.

Prolonged neutropenia has been shown to be associated with worse outcome in patients with fever and neutropenia. [24,33] In a study by Wehl and colleagues, [34] those with neutropenia lasting greater than 5 days had an increased incidence of prolonged fever, need for antibiotic modification, and most important, death from infection. Predictions of duration of neutropenia, although not perfect, can be made by the clinician based on knowledge of the patient, his or her disease, and the treatment regimen used. In pediatrics, patients undergoing therapy for AML or Burkitt's lymphoma and those undergoing induction therapy for acute lymphoblastic leukemia (ALL) clearly have treatment-related prolonged neutropenia. Patients with solid tumors undergoing high-dose chemotherapy with stem cell rescue may also have long periods of neutropenia. In addition, patients with relapsed disease with bone marrow involvement also often have prolonged neutropenia.

Comorbidity at the time of presentation has been shown to be associated with adverse outcomes in several studies. [27,29,35] Comorbidity has been variably defined but usually includes signs and symptoms related to the acute infectious episode (e.g., hypotension, tachypnea, hypoxia, or chest radiograph changes) as well as noninfectious complications of therapy (e.g., mucositis requiring i.v. narcotics).

Other factors have also shown to be predictive of outcome in patients with fever and neutropenia. Rackoff and colleagues [36] found that the risk of bacteremia in pediatric oncology patients was associated with fever greater than 39 $^{o}$C and an absolute monocyte count of less than $0.1 \times 10^{9}$ per L at the time of presentation with fever and neutropenia. Klaassen and colleagues [37] found that patients with a presenting monocyte count of greater than $0.1 \times 10^{9}$ per L and without comorbidity or an abnormal chest radiograph were at low risk for significant bacterial infections. Other authors have formed a definition of low risk based on information gathered during a period of inpatient observation. Lucas and colleagues [35] retrospectively reviewed episodes of fever and neutropenia in pediatric oncology patients. It was found that those patients presenting without signs of sepsis, who were afebrile and had an absolute neutrophil count greater than 100 at 48 hours had a low risk of serious medical complication and could be considered for early discharge. In the patients admitted with fever and neutropenia, those with negative blood cultures, no or improved focal signs of infection, and evidence of bone marrow recovery were found to be low risk. [38,39 and 40]

Based on risk stratification, there is now evidence both in adults and pediatrics that oral antibiotic therapy in the subset of patients with low risk fever and neutropenia is safe and effective. [41,42 and 43] A placebo-controlled, randomized trial of in-hospital therapy with either i.v. ceftazidime or oral ciprofloxacin and amoxicillin/sulbactam in patients with low-risk fever and neutropenia showed the oral regimen to be both safe and effective. Outpatient therapy with i.v. antibiotics in low-risk febrile neutropenic oncology patients has also been shown to be feasible. [44,45 and 46]

Shifting therapy for low-risk fever and neutropenia in pediatric oncology patients to oral therapy in the outpatient setting should be evaluated by careful study before it becomes the standard of care. To make this shift possible, practitioners must have a validated system to accurately prognosticate a pediatric cancer patient's risk of serious complication or death from infection. The length of time of inpatient observation, if any, and design of outpatient follow-up needs to be determined to ensure the efficacy and safety of such regimens. In addition, the potential burden on patients and families, satisfaction with care in the inpatient versus outpatient settings, and cost, including level of reimbursement for services and out-of-pocket expenses for patients and their families need to be assessed.

**Evaluation of Febrile Non-Neutropenic Patients**

The evaluation of a febrile non-neutropenic cancer patient should include a careful history and physical examination. Bacterial cultures of the blood should be obtained. Patients with localized symptoms or signs should undergo the appropriate diagnostic procedures—for example, aspiration for culture and Gram's stain of accessible sites of cellulitis, stool cultures in patients with diarrhea, and lumbar puncture for patients with meningeal irritation. Patients who are non-neutropenic,

clinically well, without any identifiable focus of infection, and without an indwelling central venous catheter may be observed without empiric therapy. Patients with focal findings should receive appropriate therapy based on the site involved (e.g., amoxicillin for otitis, cephalexin for cellulitis). These individuals should be followed closely to assure that the infection responds appropriately.

Patients with indwelling venous access catheters (e.g., Hickman, Broviac) who become febrile, even if they are non-neutropenic, present a special problem. The incidence of infectious complications in patients with intravascular devices can be high with central-line associated bacterial bloodstream infections being of most concern. Blood cultures should be obtained from each port of a multilumen catheter and from at least one peripheral venipuncture site. The catheter exit site should be examined carefully for signs of erythema, tenderness, or discharge, and any discharge material should be cultured. If signs of infection or clinical instability are observed, an antibiotic regimen designed to cover the most commonly encountered line-related pathogens (i.e., *Staphylococcus aureus, Staphylococcus epidermidis*, and Gram-negative aerobes) should be initiated. A broad-spectrum third-generation cephalosporin (e.g., ceftriaxone) offers adequate initial coverage in the absence of an obvious tunnel infection. Antibiotics should be continued for a 48- to 72-hour trial. If the pre-antibiotic blood and catheter culture results are negative and no site of infection is determined, the antibiotics may be withdrawn, whether or not fever persists. This allows for a thorough evaluation of the cause of fever, without the confounding influence of antibiotics. If the cultures are positive, a full therapeutic course is necessary ( Fig. 41-2).

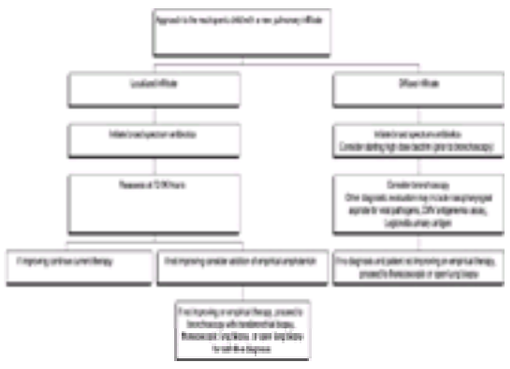


**FIGURE 41-2.** Algorithm for the management of the child with neutropenia and a pulmonary infiltrate. CMV, cytomegalovirus.

## INITIAL EMPIRIC TREATMENT OF THE FEBRILE NEUTROPENIC PATIENT

### General Considerations

The prompt initiation of empiric antibiotics when the neutropenic cancer patient becomes febrile has been the single most important advance in the management of the immunocompromised host. Before this policy was instituted, the mortality rate from gram-negative infections (especially with *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*) approached 80%.[47,48] With the widespread use of effective empiric antibiotics, the overall mortality rate has dropped to between 10% and 40% for infections caused by gram-negative bacteria.[49] What are the criteria for an empiric antibiotic regimen? Between 85% and 90% of pathogens that are documented to be associated with new fevers in immunosuppressed patients are bacteria.[24,50] Because gram-positive or gram-negative bacteria or both can be responsible, any empiric antibiotic regimen must cover a broad spectrum, provide high serum bactericidal drug levels, and be as nontoxic and as simple to administer as possible. These conditions have traditionally required the combination of two or more antibiotics. Several regimens, usually consisting of a cephalosporin, an aminoglycoside, and extended-spectrum penicillin, have been used.[49,51,52] However, no combination regimen has proved clearly superior to others. In practice, a number of options are available for the empiric management of the febrile neutropenic patient, the ultimate choice of which should depend on the predominant organisms, antibiotic sensitivity patterns, cost, and experience at the center in which the patient is being treated.

### Antibiotic Monotherapy

The development of third- and fourth-regeneration cephalosporins (e.g., ceftazidime and cefepime, respectively) and carbapenems (e.g., imipenem and meropenem) has provided alternatives to the more traditional aminoglycoside-containing combination regimens ( Table 41-2).[50,51,52,53] and [54] Several of these compounds are able to provide breadth of antimicrobial spectrum and high bactericidal levels when used as single agents. The use of antibiotic monotherapy for the empiric management of the febrile neutropenic patient is attractive because of the ease of administration, lower cost, and lesser toxicity of a single drug.

| |
|---|
| **Criteria for central venous line removal:** |
| Evidence of a local tunnel infection |
| Persistent positive blood cultures |
| Recurrent positive blood cultures with the same pathogen |
| Positive blood cultures for |
| *Candida* sp. |
| Polymicrobial infections |
| Vancomycin-resistant enterococci |
| **Criteria for probable central venous line removal:** |
| Exit-site or pocket space infection with *Pseudomonas aeruginosa* or mycobacteria |
| Clinical deterioration with known positive blood cultures |
| Positive blood cultures for |
| *Staphylococcus aureus* |
| Viridans group streptococci |
| *Bacillus* sp. |

**TABLE 41-2. MANAGEMENT OF CENTRAL VENOUS CATHETERS IN PATIENTS WITH BACTEREMIA**

The first agent to undergo significant evaluation as monotherapy was ceftazidime. The efficacy of ceftazidime as a monotherapeutic regimen was supported by the results of a prospective randomized trial at the National Cancer Institute (NCI) in which ceftazidime monotherapy was compared with the combination of cephalothin, carbenicillin, and gentamicin for the initial empiric management of 550 episodes of fever and neutropenia.[51] Overall, the results demonstrated equivalent rates of success (i.e., survival of the patient through neutropenia, with or without antibiotic modifications of the initial regimen) between ceftazidime and the combination regimen. Significantly more modifications were required among patients randomized to ceftazidime, however, reflecting the need for anaerobic coverage in patients who developed necrotizing gingivitis or perirectal cellulitis, and the greater need for vancomycin for patients with documented gram-positive infections. Patients with documented infections more frequently required changes in antimicrobial therapy than did those with unexplained fever, but the need was the same (59%) for those receiving monotherapy or combination therapy. Subsequently, a large multicenter, randomized European study that compared treatment of 876 episodes of fever and neutropenia with either ceftazidime or piperacillin plus tobramycin confirmed the efficacy as well as the reduced toxicity of ceftazidime monotherapy over the combination regimen.[54] The majority of included patients had profound and prolonged granulocytopenia resulting from acute leukemia treatment or bone marrow transplantation, and even these patients responded equally well to combination and single-agent empiric therapy, as assessed by defervescence or eradication of infecting organisms. Those receiving ceftazidime alone experienced fewer adverse events, however.

Based on these results, many centers have adopted ceftazidime monotherapy as a standard of care for the initial management of the febrile neutropenic patient. Nonetheless, several concerns have been raised regarding the use of ceftazidime monotherapy.[55] First, the relative lack of activity of third-generation cephalosporins against gram-positive cocci, particularly *S. aureus* and some strains of a-hemolytic streptococci, prompted some investigators to advocate inclusion of vancomycin in the primary regimen.[56,57] Second, some have argued for the inclusion of an aminoglycoside in the initial regimen to maximize the activity against gram-negative pathogens and to decrease the emergence of resistant organisms.

The decision about appropriate empiric regimens must ultimately be individualized for each institution. Oncology centers have different patterns of microbial isolates

and antibiotic resistance, and this must be taken into account. The 1997 recommendations (currently under revision) from the Infectious Diseases Society of America (IDSA) acknowledge this fact and emphasize the need to tailor therapy to local patterns of infection and individual patient characteristics. [58] Nevertheless, it is clear that the initial empiric management of a febrile, neutropenic cancer patient may be accomplished with a single antibiotic such as ceftazidime or imipenem. Regardless of the regimen chosen, the clinician must recognize the indications for and use appropriately the modifications essential to ensure a successful outcome ( Table 41-3).

**TABLE 41-3. COMMONLY USED ANTIMICROBIAL AGENTS FOR PEDIATRIC CANCER PATIENTS**

The use of empiric vancomycin for patients with fever and neutropenia has received special attention. The recommendations from the IDSA *1997 Guidelines for the Use of Antimicrobial Agents in Neutropenic Patients with Unexplained Fever*[58] regarding the use of vancomycin are that it should "probably" be used as empiric therapy in institutions with high rates of gram-positive organisms leading to fulminant infections (i.e., *Streptococcus viridans*, methicillin-resistant *S.epidermidis*). Empiric vancomycin is also recommended for certain patient groups, including those with obvious serious central line infections, those receiving intensive chemotherapy leading to significant mucositis (specifically, high-dose cytarabine for AML), those who have received quinolone prophylaxis before their febrile episode, patients with known colonization with organisms treatable only with vancomycin, and patients presenting with hypotension. If no infection is identified, it is recommended that vancomycin be stopped after 3 or 4 days.

## Other Antibiotics Used to Manage Immunocompromised Patients

### *Carbapenems: Imipenem and Meropenem*

Imipenem and meropenem are members of the class of b-lactam antibiotics called carbapenems. Although their mechanism of bactericidal activity involves interference with bacterial cell wall synthesis, as does that of other b-lactam drugs (e.g., penicillins, cephalosporins), imipenem and meropenem uniquely possess the broadest antimicrobial spectrum of any currently available b-lactam antibiotic. In addition to their activity against gram-negative aerobes, including *P. aeruginosa* and the Enterobacteriaceae, imipenem and meropenem act against many gram-positive organisms (e.g., *S. aureus*, enterococci, some coagulase-negative staphylococci) and most anaerobic organisms.[59]

In a large, randomized trial at the NCI, imipenem monotherapy was found to be comparable to ceftazidime monotherapy for empiric coverage in febrile neutropenic adults and children. Overall success (i.e., survival through neutropenia, with or without modifications of the initial regimen) of more than 95% occurred in both monotherapy groups. Despite the broader spectrum of imipenem, however, modifications were required as frequently by those receiving that drug as it was by those receiving ceftazidime, except for fewer additions for anaerobic coverage with imipenem. [53]

Several drawbacks of imipenem therapy have been identified that limit its use. Nausea and vomiting are well documented adverse effects of imipenem; in the NCI trial, approximately one-third of febrile neutropenic patients receiving imipenem reported significant nausea, with approximately one-third of those requiring a switch to another antibiotic. In contrast, only 2% of ceftazidime recipients reported associated nausea. Also, an increased frequency of *Clostridium difficile* colitis was observed among imipenem recipients in the NCI study, presumably related to alterations in normal anaerobic bowel flora. This has not been a commonly reported effect but is one to be considered when treating patients undergoing multiple cycles of chemotherapy. Imipenem is also known to decrease the seizure threshold in seriously ill patients and in those with central nervous system pathology, and it should be avoided in these patients. [60]

The documented efficacy of imipenem in the initial management of fever and neutropenia allows its inclusion in the array of alternative therapies, but given its toxicities, a more precisely defined role for this drug may be as initial coverage in neutropenic patients who present with a source of anaerobic infection, such as a perirectal cellulitis, marginal gingivitis, or typhlitis. Imipenem should be used cautiously in the treatment of *P. aeruginosa*, because this organism can readily become resistant to imipenem during the course of therapy. It is recommended that serious infections caused by *P. aeruginosa* be treated with the combination of imipenem plus an aminoglycoside.[61]

Imipenem is impervious to destruction by amp-C chromosomal-mediated b-lactamases that are commonly produced by *Enterobacter*, *Citrobacter*, and *Serratia* sp. Treatment of infections caused by these pathogens by ceftazidime (or another b-lactam agent) may induce b-lactamase production and, accordingly, leads to therapeutic failures.[62] Because imipenem offers the advantage of b-lactamase stability as well as efficacy against most of these organisms,[63] it is an appropriate treatment for serious infections caused by *Enterobacter* and related species, or as empiric therapy in ill patients previously treated with multiple antibiotics, in whom the likelihood of resistant organisms is increased.

Meropenem is another broad-spectrum carbapenem antibiotic that shares many of the same *in vitro* antimicrobial properties of imipenem. Meropenem has been evaluated as an agent for empiric therapy of fever and neutropenia. It has the potential advantage over imipenem of less GI toxicity and does not have the effect of altering seizure threshold. A large trial that evaluated more than 1,000 episodes of fever and neutropenia compared monotherapy with meropenem versus combination therapy with ceftazidime and amikacin and showed that the two regimes are equally effective and both well tolerated. [64] In addition, there have been several smaller studies comparing meropenem to ceftazidime alone or to imipenem, with results suggesting equivalency. [65,66] *Stenotrophomonas maltophilia* is usually resistant to imipenem and meropenem. An oxidase-negative, gram-negative bacillus causing bacteremia in a febrile neutropenic patient who is already receiving imipenem is likely to be *S. maltophilia*. Trimethoprim-sulfamethoxazole (TMP/SMX) is the preferred antibiotic against *S. maltophilia*. Multidrug-resistant *P. aeruginosa* may also emerge during the course of carbapenem therapy.

### *Cefepime*

Cefepime is a potent, broad-spectrum, fourth-generation cephalosporin with enhanced activity against gram-positive and gram-negative aerobes, including some pathogens resistant to other cephalosporins. The toxicity profile includes mild GI symptoms and neurological side effects. Cefepime has been compared to ceftazidime, imipenem, and piperacillin/tazobactam in a series of relatively small trials evaluating monotherapy for fever and neutropenia. In each of these studies the regimens were not statistically different in terms of efficacy or toxicity. [67,68] and [69]

### *Piperacillin/Tazobactam*

Tazobactam is an irreversible b-lactamase inhibitor that has been paired with the extended-spectrum piperacillin to yield a broad-spectrum agent that is relatively stable and active in the presence of many of the common b-lactamases. Its spectrum includes many of the clinically important gram-positive and gram-negative aerobes and anaerobes. This agent has been evaluated, again in a series of small trials, in combination with aminoglycosides and as monotherapy. [68,69] and [70] Larger studies are required to evaluate this agent, particularly against *P. aeruginosa* and stably derepressed b-lactamase–producing gram-negative bacilli. Pending such studies, piperacillin/tazobactam is best used in combination with an aminoglycoside in febrile neutropenic children.

### *Fluoroquinolones*

The fluoroquinolones are a group of synthetic antibiotics that possess a broad spectrum of activity, including most aerobic gram-positive and gram-negative bacteria. Ciprofloxacin, norfloxacin, and ofloxacin were the initial family of quinolones developed. The use of trovafloxacin, a newer quinolone with a broader spectrum, including improved gram-positive coverage and antianaerobic activity, was restricted when postmarketing surveillance raised concerns regarding hepatic toxicity. Levofloxacin, another novel quinolone, has gained popularity recently. It has the advantages of excellent bioavailability, twice-daily dosing, and improved gram-positive coverage, including some species of penicillin-resistant pneumococci. Other novel quinolones, including gatifloxacin and moxifloxacin are in various stages of development.

The unique mechanism by which the quinolones exert bactericidal activity and inhibition of the DNA gyrase, responsible for supercoiling and packaging of bacterial DNA, is not shared by any other class of antibiotic. Because quinolones are structurally unrelated to the b-lactams or to any other antibiotic class, cross-resistance between the fluoroquinolones and other antibiotics is rare. Fluoroquinolones are usually active against multiresistant organisms; ciprofloxacin, for example, exhibits activity against many of the resistant gram-negative rods, including *P. aeruginosa, Serratia, Enterobacter*, and *Klebsiella* sp., that are responsible for serious infections in neutropenic and otherwise immunocompromised patients. Ciprofloxacin activity against streptococcal species, including a-hemolytic streptococci (e.g., *Streptococcus mitis, Streptococcus sanguis*) and *S. pneumoniae*, is often inadequate.[71] The newer quinolones have been developed in part to overcome this deficiency.

Because of the lack of reliable streptococcal coverage, ciprofloxacin is not useful as a single agent for empiric therapy in febrile neutropenic patients, but the addition of vancomycin or a penicillin to i.v. ciprofloxacin yields a regimen that compares favorably with more traditional combinations of a b-lactam plus an aminoglycoside.[72,73] Evaluations of levofloxacin as empiric therapy for fever and neutropenia are ongoing.

Experience with the fluoroquinolones in the pediatric population has been limited by evidence of joint toxicity in experimental juvenile animals.[74] Nonetheless, ciprofloxacin is being given with increasing frequency to children with cystic fibrosis who are colonized with strains of *P. aeruginosa* and to children with other refractory gram-negative infections. A report on more than 1,700 young patients who received ciprofloxacin therapy had only a few reports of transient arthralgias that resolved promptly on discontinuation of the drug.[75,76] Extensive physical and radiographic examination, including magnetic resonance imaging (MRI), of 12 children taking ciprofloxacin for 3 months revealed no evidence of joint toxicity in one study.[77] For children with cancer who are at risk for severe infections and in whom a very potent oral antibiotic would potentiate the possibility of outpatient therapy, the benefits of fluoroquinolone therapy in selected cases may be found to outweigh the risk of joint toxicity.

### *Linezolid and Quinupristin/Dalfopristin*

These two agents have been developed in response to the need for better therapy for gram-positive pathogens, especially in regards to the emergence of vancomycin-resistant pathogens.[78] Linezolid is an oxazolidinone with a unique mechanism of protein synthesis inhibition, inhibiting the initiation complex formation. Because of its unique mode of action, it does not exhibit cross-resistance with other antimicrobials. It has excellent oral bioavailability and can be administered either orally or parenterally. In addition, it has been shown to have a favorable toxicity profile. It is bacteriostatic against methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus* sp., and penicillin- and cephalosporin-resistant strains of *S. pneumoniae.*[79,80]

Quinupristin/dalfopristin is a streptogramin antibiotic with activity against a spectrum of gram-positive organisms similar to that of linezolid.[81] The toxicity profile includes myalgias and arthralgias in approximately 10% of patients. In addition, peripheral venous infusion has been associated with local complications of pain, inflammation, and thrombophlebitis, and therefore it is recommended that it be administered via a central venous catheter. A concern with this agent is the fact that there is already reported resistance in some strains of *Enterococcus faecium* as well as staphylococci.

### *Aztreonam*

Aztreonam acts exclusively on gram-negative organisms, including *P. aeruginosa* (but not the non-*aeruginosa* pseudomonads), *Serratia, Enterobacter*, and other Enterobacteriaceae (e.g., *E. coli, Klebsiella*). The addition of an aminoglycoside to aztreonam is prudent in the treatment of serious *P. aeruginosa* infections. Because there is no gram-positive or anaerobic coverage, aztreonam should not be used as a single agent for empiric therapy in neutropenic or severely ill patients. Limited data indicate that the combination of aztreonam and vancomycin is effective for empiric coverage of febrile neutropenic cancer patients.[82]

A particular benefit of aztreonam is the apparent lack of antigenic cross-reactivity with the b-lactam antibiotics.[83] Aztreonam is most useful for patients with significant allergy to penicillin or other b-lactams in whom an antipseudomonal agent is desirable or required. The gram-negative spectrum and absence of renal toxicity allow the use of aztreonam as an alternative to aminoglycosides in certain instances.

## ANTIFUNGAL AGENTS

The armamentarium of systemically administered antifungal compounds has expanded from amphotericin B (AMB) as the only available drug to the new classes of antifungal imidazoles and triazoles, as well as lipid formulations of AMB. The antifungal and pharmacologic properties of these compounds, as they pertain to treatment of life-threatening mycoses in children, have been reviewed extensively elsewhere[84] and are summarized in the following section.

### Amphotericin B

AMB is the cornerstone of therapy in most critically ill patients with deeply invasive fungal infections. The principal mechanism of action of AMB, as of other polyenes, is the binding to ergosterol, the principal sterol present in the fungal cell membrane, which results in altered membrane permeability and causes leakage of sodium, potassium, and hydrogen ions. AMB also binds to a lesser extent to other sterols, such as cholesterol, which accounts for much of the toxicity associated with its usage. Oxidation-dependent AMB-induced stimulation of macrophages is another proposed mechanism of the chemotherapeutic effect of this polyene.

The pharmacokinetic profile of AMB in children differs from that in adults. Children older than 3 months have a smaller volume of distribution (less than 4 L per kg) and a faster clearance (greater than 0.026 L per kg per hour) than what is usually found in adults. The peak serum concentrations are significantly lower in approximately 50% of those obtained in adults receiving equivalent doses. There is a strong inverse correlation between patient age and total clearance of AMB, suggesting that higher dosages may be better tolerated in patients aged between 3 months and 9 years.

Acute or infusion-related toxicity of AMB is characterized by fever, chills, rigor, nausea, vomiting, and headache. Fever, chills, and rigors may be mediated by TNF and IL-1, cytokines that are released from human peripheral monocytes in response to the drug. These acute reactions may possibly be blunted by corticosteroids, acetaminophen, aspirin, other nonsteroidal anti-inflammatory drugs, or meperidine. Corticosteroids should be used only in relatively low dosages (e.g., hydrocortisone, 0.5 to 1.0 mg per kg). Meperidine in low doses (0.2 to 0.5 mg per kg) interdicts development of rigors; acetaminophen may decrease fever but appears to have little effect on rigors. Aspirin should be avoided in thrombocytopenic patients.

Nephrotoxicity is the most significant dose-limiting adverse effect of AMB; it may be classified as glomerular or tubular. The clinical and laboratory manifestations of glomerular toxicity include a decrease in glomerular filtration rate and renal blood flow, as evidenced by azotemia. Tubular toxicity is manifested as the presence of urinary casts, hypokalemia, hypomagnesemia, renal tubular acidosis, and nephrocalcinosis. Hypomagnesemia may be more profound in patients with cancer who develop a divalent cation-losing nephropathy associated with cisplatin. Azotemia is usually reversible, and renal function usually returns to normal levels after cessation of therapy. However, return to pretreatment levels may take several months in some cases. Administration of sodium in the form of normal saline, initially at 2 to 3 mEq per kg every 24 hours to a maximum of 4 to 6 mEq per kg every 24 hours, is often effective in preventing or attenuating the development of azotemia, possibly through inhibition of tubuloglomerular feedback. However, sodium loading requires close monitoring of patients to avoid hypernatremia, hyperchloremia, metabolic acidosis, and pulmonary edema. Furthermore, sodium loading does not ameliorate, and may indeed aggravate, hypokalemia.

Among the important drug interactions with AMB is the renal toxicity caused by aminoglycosides and cyclosporin during administration of AMB. Acute pulmonary reactions (hypoxemia, acute dyspnea, and radiographic evidence of pulmonary infiltrates) have been associated with simultaneous transfusion of granulocytes and infusion of AMB. Although some investigators have disputed the causality of AMB in such reactions, a rational approach is to separate the infusions of AMB and

granulocytes by the longest possible time period.

**Lipid Formulations of Amphotericin B**

Three carefully engineered lipid formulations of AMB have been approved in North America and Western Europe: a small unilamellar vesicle formulation of liposomal AMB (LAMB or AmBisome), AMB lipid complex (ABLC or Abelcet), and AMB colloidal dispersion (ABCD, Amphotec, or Amphocil).[85] Liposomal nystatin is an investigational compound. Based on open-label compassionate release and randomized studies, ABLC was the first lipid formulation approved in the United States by the Food and Drug Administration (FDA) in November 1995 for both children and adults. ABLC, 5 mg/kg per day i.v., in an emergency compassionate release protocol, was found to be active in treatment of immunocompromised pediatric patients with refractory mycoses and those with intolerance to conventional AMB.[86] This study found little dose-limiting nephrotoxicity of ABLC. A phase I-II study of ABLC in children with hepatosplenic (chronic disseminated) candidiasis found that the compound administered at 2.5 mg per kg for 6 weeks was effective, had no dose-limiting nephrotoxicity, and appeared to reach steady state plasma pharmacokinetics by 7 days.[87] A phase I-II study of the safety, toleration, and activity of LAMB in persistently febrile neutropenic pediatric oncology patients has recently been completed.

The introduction of lipid formulations has been an important advance in improving the therapeutic index of AMB. Because toxicity is the major dose-limiting factor of this drug, lipid formulations have been developed to reduce toxicity and permit larger doses to be administered. Although classically considered as liposomal formulations of AMB, the investigational and clinically approved formulations of AMB have a wider diversity of lipid structure. Liposomes (defined as phospholipid bilayers of one or more closed concentric structures) and other lipid formulations have been used as vehicles for AMB with encouraging results. The lipid formulation may provide a selective diffusion gradient toward the fungal cell membrane and away from mammalian cell membrane. The lipid composition, molar ratio of lipid, and liposomal size all play a role in toxicity.

Although associated with less nephrotoxicity, lipid formulations may confer their own patterns of toxicity. For example, the multilamellar lipid formulation of AMB induced reversible hypoxemia, pulmonary hypertension, and depression of cardiac output during infusion.[88] Infusion-related toxicity also may be seen with ABCD, ABLC, and LAMB. Unfortunately, the relatively greater expense of the lipid formulations of amphotericin B limits their broader use as less toxic alternatives to conventional AMB. We consider that the most appropriate use for the lipid formulations of AMB in pediatric oncology is for treatment of invasive fungal infections that are refectory to conventional therapy or for intolerance of conventional antifungal therapy. A lipid formulation of amphotericin B is also appropriate as initial empiric therapy or in definitive therapy for proven mycoses in high-risk patients receiving concomitant nephrotoxic agents (e.g., cyclosporine), those with preexisting renal impairment, those with an anticipated course of protracted neutropenia during which dose-limiting nephrotoxicity may ensue.[89]

**Flucytosine**

The mechanisms of action, pharmacokinetics, safety, antifungal properties, and clinical utility of flucytosine (5-fluorocytosine, or 5-FC) have been reviewed elsewhere.[90] 5-FC is most frequently used as an adjunct to AMB therapy. This combination was originally proposed because of the observation that AMB potentates the uptake of 5-FC by increasing fungal cell membrane permeability. Two mechanisms of action have been reported for 5-FC: 5-fluoro-deoxyuridylic acid monophosphate competitive inhibition of thymidylate synthase and 5-fluorouracil disruption of protein biosynthesis. Because of rapid emergence of resistant strains, 5-FC is used in treatment of candidiasis, aspergillosis, cryptococcosis, or other opportunistic mycoses only in combination with AMB.

Because it is a low molecular weight, water-soluble compound, orally administered 5-FC is absorbed from the GI tract rapidly and almost completely, providing excellent bioavailability. There is negligible protein binding in serum and excellent tissue penetration, including cerebrospinal fluid, with a volume of distribution that approximates that of total body water. Because 5-FC is almost completely filtered unchanged by the kidney, a decline in glomerular filtration rate results in an increase in serum concentrations of 5-FC and a higher risk of toxicity. Dose-dependent bone marrow suppression is the most serious toxicity associated with administration of 5-FC. GI side effects, such as diarrhea, nausea, and vomiting, are the most common symptomatic side effects, occurring in approximately 6% of patients. Abnormal elevation of hepatic transaminases has also been reported in approximately 5% of patients receiving the drug. Conversion of 5-FC to 5-fluorouracil by GI flora may account for most of these toxicities. These adverse effects may be controlled by close monitoring of the serum concentrations and adjustment of the dose to maintain peak serum concentrations between 40 and 60 mg per L. Because 5-FC is used in combination with AMB, the conventional dosage of 150/per kg per day is not recommended in most patients. Instead, 100 mg/kg per day is a preferable starting dose in patients with normal renal function. As the glomerular filtration rate decreases as a result of AMB, the 5-FC dosage is reduced to less than 100 mg/kg per day in three to four divided doses. However, some children may have unusually high clearance rates and require doses of 150 mg/kg per day or more.

**Antifungal Azoles**

The antifungal azoles include imidazoles (clotrimazole, miconazole, and ketoconazole) and triazoles (itraconazole and fluconazole). Clotrimazole and miconazole are available in topical applications. The parenteral formulation of miconazole is no longer available. The use of ketoconazole has been generally supplanted by itraconazole and fluconazole in the pediatric oncology setting. Thus, only fluconazole and itraconazole are discussed here.

The antifungal azoles are synthetic compounds that demonstrate less toxicity than AMB, have flexibility for oral administration, and have comparable efficacy against certain infections. The antifungal azole agents function principally by inhibition of the fungal cytochrome P-450 enzyme lanosterol 14a-demethylase, which is involved in the synthesis of ergosterol.

***Fluconazole***

Fluconazole is a water-soluble meta-difluorophenyl bis-triazole and is available in both oral and parenteral formulations. Fluconazole has been shown to be effective against infections caused by *Candida* sp., *Cryptococcus neoformans*, and other fungi in patients with neoplastic diseases, human immunodeficiency virus (HIV) infection, and other immunocompromised states.

Fluconazole is a relatively small molecule with rapid absorption and excellent bioavailability. Fluconazole is only weakly bound to serum proteins (12%). The concentration–time curves of orally and parenterally administered fluconazole are almost superimposable. Fluconazole exhibits linear plasma kinetics and is only slightly metabolized. In the setting of renal impairment, the dosage of fluconazole is adjusted to reflect glomerular filtration. A 50% reduction of dosage is recommended in patients with a creatinine clearance of 21 to 50 mL per min, and a 75% reduction in those with a clearance of less than 21 mL per min. Oral absorption of fluconazole does not depend on a low intragastric pH, feeding, fasting, or mucosal integrity.

A study conducted at the NCI and Children's National Medical Center found that the plasma half-life of fluconazole in children aged 5 to 15 years was substantially reduced in comparison with the half-life in adults (17 hours in children versus reports of 27 and 37 hours in adults).[91] In light of this more rapid clearance of fluconazole, life-threatening fungal infections in children are treated with 12 mg per kg in two divided doses (assuming normal renal function) to approximate the dosage equivalency in adults, as defined pharmacokinetically by the area under the concentration–time curve.

Fluconazole penetrates well into cerebrospinal fluid.[92] This distribution property results in cerebrospinal fluid to serum concentration ratios of between 0.5 and 0.9, increasing to between 0.8 and 0.9 in the setting of meningeal disease. Fluconazole has been well tolerated with very few dose-limiting side effects in different pediatric populations. Nausea, other GI symptoms, and elevated hepatic transaminases occur infrequently and are usually reversible. Fluconazole does not appear to affect the synthesis of steroid hormones.

The drug interactions of fluconazole in principle are similar to those of other azoles. For example, fluconazole has been reported to precipitate phenytoin toxicity because of inhibition of metabolism, thus warranting monitoring of phenytoin concentrations during coadministration of fluconazole. Concentrations of cyclosporine may be increased, and the effects of warfarin may be potentiated.

***Itraconazole***

Itraconazole, although structurally similar to ketoconazole, has a broader spectrum of antifungal activity, less toxicity, a longer plasma half-life, and the capacity to penetrate into brain tissue. The spectrum of itraconazole includes *Candida* sp., *C. neoformans*, *Trichosporon* sp., *Aspergillus* sp., dematiaceous molds, and the endemic dimorphic fungi, including *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, and *Paracoccidioides brasiliensis*. Despite this extended

spectrum and greater safety profile, itraconazole has limited bioavailability. Itraconazole is soluble only at low pH, as in the normal gastric milieu. There is wide intersubject variation in the plasma concentration curves of itraconazole, particularly in patients receiving intensive cytotoxic chemotherapy, oral antacids, or $H_2$ receptor–blocking agents.

Plasma levels of itraconazole are substantially decreased by antacids and by $H_2$ receptor–blocking agents (i.e., cimetidine or ranitidine) because of elevated gastric pH, which impairs absorption of the drug. This erratic bioavailability compromises the role of itraconazole in neutropenic patients, particularly those with chemotherapy- or radiotherapy-induced mucosal disruption.

Interpatient variation in absorption of itraconazole is improved by incorporation of the molecule into cyclodextrin. The cyclodextrin formulation of itraconazole is approved for treatment of mucosal candidiasis. Higher dosages of hydroxypropyl cyclodextrin itraconazole required to treat deeply invasive infections such as aspergillosis, however, may cause GI distress in some patients due to the cyclodextrin formulation, thereby necessitating the use of capsules. Thus, plasma concentrations of itraconazole should be monitored in treatment with patients with deeply invasive mycoses to assure adequate bioavailability.

Attainment of adequate plasma concentrations is critical for optimal antifungal effect of itraconazole. [93] Because itraconazole is highly protein bound (greater than 99%), with only 0.2% available as free drug, concentrations in body fluids equivalent to body water, such as saliva and cerebrospinal fluid, are negligible. Tissue concentrations, however, including those of the central nervous system, are two to five times higher than those in plasma, and they persist for longer, explaining the efficacy of the drug despite low plasma concentrations. Itraconazole is extensively metabolized by the liver to hydroxy-itraconazole, which also possesses intrinsic antifungal activity. Less than 1% of the active drug, and approximately 35% of the inactive metabolites, are excreted in the urine. Because the primary route of excretion is the biliary tract, no adjustment of dosage is necessary in patients with renal impairment.

Itraconazole has properties of drug interaction with cyclosporin, rifampin, phenytoin, phenobarbital, antihistamines, coumadin, and oral hypoglycemic agents. Important drug interactions between itraconazole and other agents can prolong the plasma half-life of cyclosporin, which may lead to cyclosporin-induced nephrotoxicity. Consequently, serum cyclosporin levels should be closely monitored, and dosages of cyclosporin should be adjusted in patients receiving itraconazole. Itraconazole's inhibition of the metabolism of antihistamines such as terfenadine and astemizole may lead to widening QT intervals and cardiac ventricular arrhythmias, including torsade de pointes. The serum concentrations of itraconazole may be markedly decreased with concomitant administration of drugs that induce hepatic microsomal enzymes, such as rifampin and phenobarbital. Caution should also be exerted in the coadministration of traconazole with vinca alkaloids, coumadin, and oral hypoglycemic agents, because the increased concentrations of these drugs may cause increased neuropathy, prothrombin time, and hypoglycemia, respectively.

Itraconazole is well tolerated with long-term use. Itraconazole has a relatively low incidence of hepatic toxicity (less than 5%). Most of the reported adverse reactions are transient; they include GI disturbances, dizziness, and headache, and no adverse effect on steroidogenesis.

A parenteral formulation of itraconazole in hydroxypropyl cyclodextrin solution has recently become available. This encouraging development obviates the problems of bioavailability and tolerance of the oral formulations. There are, however, limitations to the use of this new parenteral formulation. Due to the potential nephrotoxicity of the vehicle, use of the command is not approved beyond 2 weeks. The formulation is supplied only in 200-mg bags that cannot be combined for use in higher dosages. More data are required in understanding the use of the hydroxypropyl cyclodextrin solution of itraconazole in treatment of invasive mycoses.

The role of combination antifungal therapy with AMB and antifungal triazoles is controversial but frequently arises in the management of the patient with refractory aspergillosis, when the combination of AMB plus itraconazole is contemplated. Because there are clinical and experimental data demonstrating antagonism of antifungal activity when amphotericin is combined with an azole, the safety and efficacy of combination therapy are most appropriately determined from a clinical trial. To date, limited data exist from a recently completed multicenter clinical trial of fluconazole versus fluconazole plus AMB in non-neutropenic patients with candidemia.

New classes of antifungal agents also have recently been introduced into clinical trials. These include second-generation triazoles (voriconazole, posaconazole, and ravuconazole) and echinocandins [caspofungin (MK0991), FK463, and V-echinocandin (LY303366)]. The second-generation triazoles have expanded antifungal spectra and potent *in vitro* and *in vivo* antifungal activity. Voriconazole and ravuconazole are formulated for both oral and parenteral administration. Echinocandins are cyclic lipopeptides that inhibit 1,3-b- D-glucan synthase activity, resulting in inhibition of fungal cell wall biosynthesis. The current generation of echinocandins has activity against *Candida* sp., *Aspergillus* sp., and *P. carinii*; however, they do not have activity against *C. neoformans.* The echinocandins are formulated only for parenteral administration.

## ANTIVIRAL AGENTS

The herpesviruses, including herpes simplex virus (HSV), CMV, and varicella-zoster virus (VZV), commonly cause infections in immunocompromised patients. Often acquired early in childhood, these viruses cause acute infections and are then maintained indefinitely in a state of latency, within dorsal root ganglia in the case of HSV and VZV, and probably within monocytes in the case of CMV. It is thought that latent herpesviruses are held in check and prevented from frequent reactivation by the presence of effective cellular immune function. Immunosuppression, as a consequence of either cancer chemotherapy or the underlying malignancy itself, permits reactivation of herpesvirus replication. Reactivation of viral replication from latency may be detected simply as asymptomatic shedding of virus, or it may manifest as invasive disease. Herpes stomatitis, localized or disseminated zoster, and interstitial pneumonitis are the most common clinical manifestations of HSV, VZV, and CMV reactivation, respectively. Several important antiviral agents have become standard components of the antimicrobial armamentarium over the last decade, and others have been added more recently.

In addition to the herpes viruses, common respiratory viruses have been increasingly recognized as significant pathogens in cancer patients. Respiratory syncytial virus (RSV), parainfluenza, and influenza have been associated with significant morbidity and mortality, particularly in patients with leukemia and those undergoing bone marrow transplantation. Agents used for the prevention and treatment of these infections are discussed below.

### Acyclovir

Acyclovir was the first widely used antiviral agent, and it has become an essential element in the supportive care of children and adults with cancer. Acyclovir is a guanine nucleoside analog that, when triphosphorylated, is selectively recognized by viral DNA polymerase as a nucleotide. Acyclovir triphosphate acts as an inhibitor of herpesvirus DNA polymerase and stops viral DNA synthesis. Because of low affinity for cellular DNA polymerases, acyclovir triphosphate has an excellent therapeutic ratio. The selective antiviral action of acyclovir and other similar compounds is caused by preferential phosphorylation (i.e., activation of the drug) by the virus-encoded thymidine kinase enzyme.

Acyclovir is effective for prophylaxis and treatment of both primary infections and reactivations of HSV types 1 and 2 in immunocompromised patients. [94,95] It is used prophylactically in seropositive persons who are undergoing intensive therapy or bone marrow transplantation. There is also evidence that it prevents reactivations of VZV and CMV in bone marrow allograft recipients who receive it prophylactically, although acyclovir itself has no therapeutic efficacy against established CMV disease.[96] Treatment of localized zoster using i.v. acyclovir in immunocompromised patients prevents dissemination and reduces mortality from visceral VZV. [97] In immunocompetent children with chickenpox, oral acyclovir marginally reduces the duration of symptoms, but i.v. acyclovir remains the standard of care in those who are immunocompromised. The i.v. doses of acyclovir required to treat VZV disease (500 mg per $m^2$ every 8 hours) are double those used for HSV therapy (250 mg per $m^2$ every 8 hours), because VZV is relatively less sensitive to the drug. Acyclovir is associated with very few adverse effects, although high i.v. doses should be given with adequate hydration to avoid renal toxicity.

### Ganciclovir

Ganciclovir is a deoxyguanine nucleoside analog, and although it is active against the all of the herpesviruses affecting immunocompromised patients (HSV, VZV, and CMV), it is much more potent than acyclovir against CMV. Ganciclovir, like acyclovir, requires activation through phosphorylation, and the triphosphate form of the drug interferes with viral DNA synthesis. The significant myelotoxic effects of ganciclovir preclude its routine use to treat HSV or VZV. It is used almost exclusively for treatment and prevention of disease caused by CMV. For invasive CMV disease (i.e., retinitis, colitis, pneumonitis), ganciclovir induction therapy at 5 mg per kg twice daily (b.i.d.) for 2 to 4 weeks is often followed by a prolonged maintenance therapy period, especially in patients with persistently impaired immune systems [e.g., patients with the acquired immunodeficiency syndrome (AIDS)].

### Foscarnet

Unlike acyclovir and ganciclovir, foscarnet does not require phosphorylation to become active. It directly inhibits the DNA polymerases of the herpesviruses. The unique function of foscarnet has made it particularly useful for the treatment of infections caused by HSV and VZV that have become resistant to the standard nucleoside analogs. Typically, these resistant viruses have, by mutation, lost the viral thymidine kinase activity that normally activates acyclovir and ganciclovir. Foscarnet has assumed an important role as a highly effective treatment for mucocutaneous lesions of chronic zoster and HSV, often associated with resistant viruses, and for CMV retinitis in patients with AIDS, in whom it may confer a slight survival advantage over ganciclovir therapy. [98] The potential benefits of the drug must be balanced against the inconvenience of foscarnet administration, which is solely by the central venous route and is on a daily basis, usually over several hours. In addition, significant renal toxicity, with decreased glomerular filtration and rising creatinine, is seen in most patients receiving foscarnet.

### Famciclovir and Valaciclovir

Famciclovir and valaciclovir are very similar to acyclovir in their structure and activity. However, each has a pharmacokinetic profile that allows for less frequent oral dosing for treatment of HZV. Twice-daily dosing of famciclovir or thrice-daily dosing of valaciclovir has clear advantages for the patient when compared with the five times daily oral acyclovir dose that is recommended for immunocompetent adults. Both agents appear to be as efficacious as acyclovir in these patients. As yet, there have been no published studies of these drugs in immunocompromised children, although there is no reason to believe that these agents would be any less effective than acyclovir. They are attractive antibiotics because of their excellent pharmacokinetic profiles and less cumbersome dosing schedules.

### Sorivudine

Sorivudine, or BVaraU, is a synthetic deoxythymidine nucleoside analog with *in vitro* activity against VZV that is approximately 1,000-fold greater than that of acyclovir *in vitro*. It is orally available, and pharmacokinetics in adults favor once-daily dosing. In a study of adult patients with HIV and zoster, oral sorivudine administered once a day compared favorably to oral acyclovir administered 5 times per day in terms of the rate of cutaneous healing. [99] Sorivudine has a significant drug-drug interaction with the chemotherapy medication, 5-fluorouracil, with concomitant administration putting individuals at significant risk for severe myelotoxicity. [100] Because of this serious drug interaction, sorivudine has not been licensed in the United States.

### Ribavirin

Ribavirin is a synthetic virostatic nucleoside with antiviral properties *in vitro* against a variety of RNA and DNA viruses. It is a small-particle aerosol usually given in a dose of 20 mg per mL in 300 mL of distilled water nebulized in an oxygen hood, tent, or mask over 12 to 18 hours for every 24-hour period. Shorter-duration therapy with high-dose aerosolized ribavirin appears to be efficacious and is more convenient. [101] Ribavirin is associated with few side effects; nausea, headache, and bronchospasm occur at low frequency. Accumulated data have suggested that health care workers are not at significant risk for adverse effects with the minimal exposure that occurs during care of a child receiving aerosolized ribavirin, although pregnant women are advised to avoid areas in which ribavirin therapy is administered because of concerns about the uncertain teratogenic potential of the drug in humans. [102]

The use of ribavirin for treatment of RSV pneumonia was originally studied in infants with severe disease. The efficacy of this drug has shown mixed results, with some studies showing improvement in overall severity of illness [103] and others showing no difference [104,105] or worse outcome in the ribavirin-treated group. [106] The ambiguity of the data prompted the American Academy of Pediatrics to change its recommendation from ribavirin “should be used” to “may be considered” for selected infants and young children at high risk for serious disease. [107] These include children with chronic lung disease, congenital heart disease, prematurity, and those who are immunosuppressed.

The mortality rate from RSV pneumonia in adults undergoing therapy for AML and in bone marrow transplant patients is high. [108] The data regarding the efficacy of ribavirin therapy in these patients is somewhat limited and is based primarily on reports of on-case series compared to historical controls. [109] There is a suggestion from the data that the early initiation of ribavirin therapy, often given in conjunction with immune globulin, may have some beneficial effect.

Intravenous ribavirin in conjunction with interferon alpha-2b is now the standard of care for patients with chronic hepatitis C infection. [110] This combination has been shown to increase sustained response rates to 40% in those individuals who are treatment naive and to 50% in those who have relapsed after initially responding to interferon alone. Response is dependent to some degree on viral genotype, with 60% of those individuals with genotype non-1 having sustained responses compared with 30% in those individuals with genotype 1. [111] This therapy is unfortunately associated with significant side effects, most notably flulike symptoms from the interferon and dose-related hemolytic anemia from ribavirin, making it intolerable for a subset of patients.

### Synergis and Respiratory Syncytial Virus Immune Globulin

Synergis is a monoclonal antibody directed at the F glycoprotein of RSV, a surface protein highly conserved among RSV isolates. It was licensed by the FDA in 1998 for the prevention of RSV in premature infants and in those with chronic lung disease. It is given monthly during the RSV season at a dose of 15 mg per kg, administered intramuscularly. With this regimen it was shown in a randomized, placebo-controlled trial involving 1,502 patients with chronic lung disease who were younger than 24 months or in patients with a gestational age less than 35 weeks and were younger than 6 months that prophylaxis during the RSV season decreased hospitalization, intensive care unit days, and severity of disease. [112] RSV can be a serious pathogen in oncology patients, especially in those with acute leukemia and those undergoing bone marrow transplantation. However, to date there are no data in the utility of this agent for prophylaxis in these patient groups.

RSV immune globulin is blood product prepared from donors selected for high titers of RSV neutralizing antibodies. The range of RSV antibody titer is 1:2,400 to 1:8,000, whereas unselected immune globulin usually has anti-RSV antibody titers of less than 1:1,000. It was licensed by the FDA in 1996 also for the prevention of RSV pneumonia in premature infants and for those with chronic lung disease. It is given once a month at a dose of 750 mg per kg i.v. Similar prophylaxis studies were performed and undertaken for synergism with similar results. Again, however, there are no data evaluating this agent for prophylaxis in oncology patients.

There has been some evaluation of RSV immune globulin for therapy for RSV disease. In a study of infants with RSV pneumonia there was no difference in outcome. [113] There are a number of small case series in adult oncology and bone marrow transplant patients using RSV immune globulin in combination with ribavirin for the treatment of severe lower tract disease, with a suggestion of improved outcomes over historical controls. [109,110]

### Amantadine and Rimantadine

Amantadine and rimantadine both have activity against influenza A but not against influenza B at clinically relevant doses. They appear to inhibit the uncoating of the viral RNA within host cells, ultimately blocking viral replication. Both drugs are well absorbed orally and are usually well tolerated, with no serious organ toxicities. The most common side effects of amantadine and rimantadine are mild GI discomfort, including loss of appetite and nausea. However, amantadine is associated with central nervous system effects such as nervousness, lightheadedness, difficulty concentrating, and insomnia, particularly in older adults. Rimantadine causes fewer central nervous system effects.

Both drugs have proved effective for prophylaxis and treatment of influenza A infections in immunocompetent patients, and there are reported successes of amantadine in immunocompromised patients with influenza A pneumonia. Although the improvements in symptoms and in viral shedding that are seen in patients treated with amantadine or rimantadine are better than those seen with aspirin or placebo treatment, they are modest, and there have been no trials in immunocompromised patients or in patients with life-threatening influenza A. [115] Wild-type viruses are usually susceptible to both drugs, but resistance (and cross-resistance) emerges rapidly when they are used clinically. Resistant influenza A virus has been isolated from children receiving rimantadine treatment and in family members receiving postexposure prophylaxis. [116] This finding is of concern, and for this reason it is suggested that simultaneous therapy and prophylaxis in the same household be avoided. Amantadine doses of 2.2 to 4.4 mg per kg b.i.d. up to 150 mg per day are suggested for young children (aged 1 to 10 years), and older children may receive 100 mg b.i.d. for prophylaxis or treatment of influenza A.

### Oseltamivir and Zanamivir

Oseltamivir and zanamivir are potent and specific neuraminidase inhibitors. *In vitro* each has been shown to have activity against both influenza A and B. Oseltamivir is administered orally, whereas zanamivir is administered intranasally. When administered early in the course of influenza A or B infection, both agents have been shown to decrease the duration of illness and the severity of symptoms.[117,118 and 119]

To date, the evaluation of the efficacy of these agents in immunocompromised individuals has not been reported. Given the data in immunocompetent individuals, it is not unreasonable to consider these agents for prophylaxis of high-risk patients with significant exposures. Zanamivir has been studied in children older than 5 years, with similar results in terms of efficacy and tolerability as those found in adult studies.[120] Oseltamivir has not been evaluated in pediatric patients.

## ANTI–*PNEUMOCYSTIS* PNEUMONIA AGENTS

There are a number of agents in common usage for the prophylaxis and treatment of PCP. Much of the data on their efficacy have been evaluated in patients with AIDS; however, there is also a substantial amount of data in oncology and bone marrow transplant patient populations.

### Trimethoprim-Sulfamethoxazole

In 1977 Hughes et al. demonstrated that prophylaxis with TMP/SMX was highly effective in preventing PCP in high-risk oncology patients.[121,122] The use of PCP prophylaxis has since become a routine part of the management of most childhood cancers. The recommended prophylactic regimen is TMP/SMX with 150 mg TMP per $m^2$ per day and 750 mg per $m^2$ per day of SMX given orally in divided doses b.i.d. during 3 consecutive days per week. Most oncology patients are able to tolerate TMP/SMX; however, side effects including bone marrow suppression and rash sometimes make alternative therapy necessary.

### Dapsone

Dapsone is a synthetic sulfone that has been shown to be effective in the treatment and prevention of PCP.[123] It acts through the inhibition of folic acid synthesis in susceptible organisms. For prophylaxis it is administered at a dose of 2 mg per kg per day. Adverse effects include rash, anemia, methemoglobinemia, agranulocytosis, and hepatic dysfunction. Dapsone has been recommended in the 1997 *USPHS/IDSA Guidelines for the Prevention of Opportunistic Infections in Persons with Human Immunodeficiency Virus* as the PCP prophylaxis for those patients unable to tolerate TMP-SMX.[124]

### Pentamidine

Pentamidine, in its aerosolized form, is another agent that has been studied extensively in both children and adults with HIV as a prophylactic agent for PCP. It has been shown to be an effective regimen when administered at a dose of 300 mg via Respirgard inhaler (Marquest, Englewood, CO) monthly. In small children it is often considered difficult to administer this drug because of the mechanics of the inhaler therapy. It has, however, been shown to be feasible, at least in a study situation, in children as young as 4 months.[125] Intravenous pentamidine has only undergone limited evaluation as a preventive regimen and was associated with a significant number of adverse drug reactions.[126]

### Atovaquone

Atovaquone is a 1,4-hydroxynapthoquinone with broad anti-protozoal activity, including proved efficacy against *P. carinii*. It has been shown to be and effective agent in treating PCP.[127,128] More recently, atovaquone was shown to be as effective as dapsone for PCP prophylaxis in patients with AIDS who were intolerant of TMP-SMX. The side effect profile of atovaquone is favorable, with the most common adverse effect being mild upper GI symptoms and diarrhea.

The drug is significantly more bioavailable in suspension form and therefore is routinely given in that form. Pharmacokinetic studies have been completed in children[129] suggesting dosing of children aged 0 to 3 months and older than 24 months at 30 mg per kg per day and for those aged 3 to 24 months, 45 mg per kg per day. It is dosed once daily. One drawback of atovaquone is that it is significantly more expensive than other oral agents when used for prophylaxis.

## MANAGEMENT OF UNEXPLAINED FEVER IN THE NEUTROPENIC PATIENT

### Duration of Antibiotic Therapy

Once antibiotics have been started empirically, there is the question of how long to continue them if a site of infection has not been defined. Low-risk patients do well if antibiotics are continued until the granulocyte count recovers to approximately 500 cells per $mm^3$.[24] Alternatively, there is increasing evidence that antibiotics may be discontinued in these low-risk patients if their neutrophil counts are rising, even though they have not risen above 500 cells per $mm^3$.[38] In a prospective study of 131 children with fever and neutropenia hospitalized for i.v. antibiotic treatment, 70 had their antibiotics discontinued and were discharged after they met the following criteria: afebrile for 24 hours, appeared clinically well, had negative cultures for a least 48 hours, exhibited control of local infection, and had evidence of bone marrow recovery for at least 1 day, as measured by rising absolute neutrophil or PMN count or platelet count. Only 1 of the 70 children required readmission for recurrent fever, whereas six of eight patients were inadvertently discharged without signs of marrow recovery and required readmission. Substantial savings in hospital costs were estimated for early discharge patients, and this approach is a reasonable option for those patients who fit specific low-risk criteria.[39] However, these guidelines do not apply to high-risk patients who remain neutropenic for more than 1 week and who do not demonstrate evidence of bone marrow recovery. Stopping antibiotic therapy too early can lead to clinical deterioration in patients who remain granulocytopenic, particularly if they are persistently febrile.

The management of the high-risk subset of patients with prolonged neutropenia was addressed in a series of prospective clinical studies that stratified them according to whether they had defervesced after the initiation of broad-spectrum antibiotics or remained persistently febrile ( Fig. 41-3).[33] Among patients who had defervesced on therapy, 41% again became febrile within 3 days of stopping antibiotics on day 7; new bacterial isolates from those with documented infections were sensitive to the antibiotics that had been withdrawn. No subsequent infections were observed among patients who continued antibiotics, however.

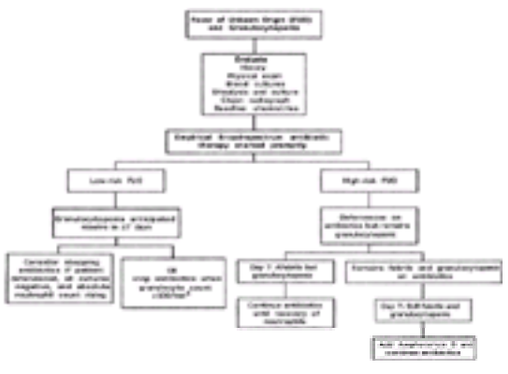


**FIGURE 41-3.** Algorithm for the initial management of the child who has unexplained fever and neutropenia. (See text for details.)

### Empiric Antifungal Therapy

The situation is more complicated for patients who remain persistently granulocytopenic and febrile despite antibiotic therapy. In a randomized clinical trial, we observed that 56% of patients with unexplained fever who remained febrile after receiving empiric antibiotics developed complications within 3 days of stopping therapy.[130] Of these, 38% became hypotensive. Simply continuing antibiotics in the face of persistent fever and granulocytopenia was also not satisfactory, however, because 31% of these patients eventually developed invasive fungal infections. These fungal infections were probably related to continued antibiotic therapy and

protracted granulocytopenia.

The background and rationale for the empiric use of an antifungal compound are based on several lines of reasoning. First, antemortem diagnosis of invasive fungal disease is difficult in an immunocompromised host. Second, withholding antifungal therapy pending a definitive diagnosis may allow dissemination to occur before the institution of therapy. Third, the outcome of a fungal infection in an immunocompromised patient is improved by early institution of therapy. Fourth, it is possible to identify patients who are at greatest risk for invasive mycoses. Neutropenic patients who remain febrile despite a 4- to 7-day trial of broad-spectrum antimicrobial therapy are particularly prone to fungal disease. [131] The use of empiric antifungal therapy would be expected to provide a dual benefit: the suppression of the fungal overgrowth that inevitably accompanies broad-spectrum antimicrobial therapy and the early treatment of subclinical, localized mycotic disease.

A prospective, randomized trial performed by the European Organization for Research on Treatment of Cancer corroborated the benefit of empiric AMB therapy for persistently febrile neutropenic patients. In that study, there were six documented fungal infections, four of which were fatal, among 64 patients who did not receive antifungal therapy, compared with only one fungemia and no deaths among 68 patients treated empirically with amphotericin (0.6 mg per kg per day or 1.2 mg per kg every other day) on or after day 4 of broad-spectrum antibiotic therapy. [132]

The point at which antifungal therapy was initiated varied in these studies. The arbitrary designation of day 7, used in the NCI trials, avoids the overuse of antifungal agents in patients who are slow to defervesce with empiric antibiotics and those who recover their granulocyte counts before day 7. Despite theoretical and clinical evidence substantiating the efficacy of empiric antifungal therapy, the toxicity of AMB limits the utility of empiric use of this compound. Less toxic alternatives are desirable.

A multicenter study, therefore, investigated whether liposomal AMB may be used instead of conventional AMB for empiric antifungal therapy in a randomized, double-blind trial design of LAMB versus conventional AMB in neutropenic children and adults with persistent fever despite broad-spectrum antibiotics. [133] Among 687 randomized patients, the composite success rate was equivalent (50% LAMB vs. 49% AMB) and independent of administration of antifungal prophylaxis or use of colony-stimulating factors. Comparable responses were observed in LAMB versus AMB, respectively, for survival rate (93% vs. 90%), resolution of fever during neutropenia (58% vs. 58%), and premature withdrawal of study drug due to toxicity or lack of efficacy (14% vs. 19%). There were fewer proved breakthrough fungal infections in patients treated with LAMB versus AMB [11 (3.2%) vs. 27 (7.8%) ($p$ = .009)]. There were fewer ($p$£.01) infusion-related fevers (17% vs. 44%), chills/rigors (18% vs. 54%), and cardiorespiratory events (dyspnea, hypotension, tachycardia, hypertension, and hypoxia; 13.1% vs. 45.6%) for LAMB versus AMB, respectively. There also was reduced ($p$ <.001) nephrotoxicity in patients treated with LAMB (19%) versus AMB (34%). Thus, this study conduced LAMB was equivalent to AMB in therapeutic success for empiric antifungal therapy in neutropenic patients, but superior in reducing proven treatment-emergent fungal infections, infusion-related toxicity, and nephrotoxicity.

For patients who remain neutropenic, antifungal therapy should be continued until the resolution of granulocytopenia. Persistence or recrudescence of fever should prompt a meticulous investigation for nonfungal infectious causes (e.g., bacterial or viral superinfections) or for a fungus that is resistant to doses of AMB given for empiric coverage (e.g., *Aspergillus* sp., *Trichosporon*, *Fusarium* sp., *Pseudallescheria boydii*, and *Scedosporium* sp.). Patients who develop a documented fungal infection should be treated with higher dosages of AMB or be enrolled onto emergency compassionate release of an investigational antifungal compound.

As lipid formulations of AMB are more costly than conventional AMB, targeting the highest-risk patients who may benefit from empiric AMB is important. Such patients include those with preexisting renal insufficiency, concomitant nephrotoxic agents, and anticipated protracted neutropenia. A recent pharmacoeconomic analysis investigated the impact of nephrotoxicity on total cost of hospitalization and the cost/benefit ratios of AMB and LAMB. [134]

## EVALUATION AND MANAGEMENT OF DOCUMENTED INFECTIONS

### Bacteremia

Approximately 10% to 30% of all febrile neutropenic cancer patients are bacteremic at presentation. [47,51] In the low-risk subgroup, the rate is consistently less than 10%.[36,37] Until the late 1970s, gram-negative aerobic organisms (especially *P. aeruginosa, E. coli*, and *K. pneumoniae*) were the most frequently isolated pathogens. Subsequently, the pattern of infections has shifted, and gram-positive bacteria are now isolated more often than gram-negative bacteria at most cancer centers. It is hypothesized that this shift is secondary to increased used of indwelling central venous catheters, fluoroquinolone prophylaxis, and high-dose chemotherapy induced oral mucositis.

The gram-positive pathogens most commonly isolated include *S. aureus, S. epidermidis, Streptococcus* sp. (including a-hemolytic streptococci), and *Enterococcus* sp. Species of *Corynebacterium* (e.g., *C. jekeium, C. diphtheriae*, and *C. equi* ) and *Bacillus* sp. are less frequently isolated and tend to occur in patients with long episodes of granulocytopenia or those with indwelling vascular access devices, respectively. [135] *E. coli, K. pneumoniae*, and *Enterobacter* sp. are now the most frequently isolated gram-negative bacilli, although more resistant species (e.g., non- *aeruginosa* pseudomonads, and *Serratia marcescens*) are occasionally encountered.

Resistance patterns in pathogens isolated from febrile neutropenic patients have emerged as a significant challenge. *Enterococcus* sp. that are resistant to vancomycin, ampicillin, aminoglycosides, or all of these, have been increasingly noted in recent years and are associated with high mortality in immunocompromised patients.[136,137] Someisolates of viridans streptococci are resistant to penicillin and cephalosporins and, therefore, require vancomycin for therapy. *S. pneumoniae* isolates with either intermediate- or high-level penicillin resistance are now relatively common in many parts of the United States. This is of special concern for immunocompromised individuals at high risk for pneumococcal septicemia—for example, asplenic patients and patients post–bone marrow transplantation with chronic graft-versus-host disease. In addition, gram-negative bacilli resistant to quinolones or cephalosporins are an increasing problem at some institutions.

In general, the morbidity and mortality rates associated with infections with gram-positive bacteria, especially the coagulase-negative staphylococci, are lower than those caused by gram-negative pathogens. An important exception is bacteremia caused by a-hemolytic streptococci, most commonly *S. mitis* and *S. sanguis*. Alpha-hemolytic streptococcal sepsis may cause sudden onset of hypotension, with progression in approximately one-fourth of cases to a syndrome that can include shock, respiratory failure due to adult respiratory distress syndrome, acute renal failure, and neurologic manifestations. Palmar erythema and subsequent desquamation may also be a feature of this syndrome. Although there is considerable variability between centers of mortality related to viridans streptococcal sepsis, the median death rate is approximately 10%.[138] The antecedent administration of high-dose cytosine arabinoside is a strongly correlative risk factor for the development of a-hemolytic streptococcal sepsis.[139] A number of other distinct risk factors have been associated with this syndrome, including the presence of mucositis, the administration of antacids or $H_2$-blockers, and prophylactic treatment with TMP-SMX or fluoroquinolone antibiotics, both of which allow for breakthrough growth of a-hemolytic streptococci.[47,140]

The most important therapeutic intervention for patients ultimately shown to be bacteremic is the prompt initiation of empiric antibiotic treatment at the time of the patient's presentation with fever and neutropenia. Necessary modifications of the initial regimen should be based on the antimicrobial sensitivity pattern of the bloodstream isolate (Table 41-3) while maintaining broad empiric coverage.

### Catheter-Associated Bacteremia

With the increased use of indwelling venous access devices, catheter-associated bacteremic episodes have become more frequent. [141] The strict diagnosis of a catheter-related versus non–catheter-related bacteremia is often difficult. Positive blood cultures drawn through a venous catheter can be considered to have arisen from one of three possible sources: colonization of the line from external sources (skin and catheter hub), hematogenous seeding of the catheter from internal sources and, rarely, contaminated infusions (e.g., platelets with bacterial contamination). Evidence of catheter-related infection as opposed to bacteremia from other sources includes a greater number of colony-forming units per mL of blood from cultures of the central line compared with simultaneous peripheral cultures and positive catheter tip cultures when the line is removed for presumed infection.

The majority of patients with fever and neutropenia and a central line–associated bacteremia do not need to have their catheters removed [142]; however, there are certain clinical situations and infectious pathogens that require line removal for cure of infection. *S. aureus* catheter-related bacteremia can generally be treated without line removal.[143] Patients with vancomycin-resistant enterococcal infections often require catheter removal for cure. Certain pathogens that are sensitive to therapy (e.g., some *Bacillus* sp.) require catheter removal to cure the infection. Patients with polymicrobial catheter-related bacteremia and those with candidemia

should have their catheters removed.

Most catheter-associated infections caused by coagulase-negative staphylococci can be controlled without removal of the catheter. [141] In addition, many gram-negative catheter infections can be treated with i.v. antibiotics and without removal of the catheter. In patients with double- or triple-lumen catheters, the antibiotic infusions should be rotated among each of the catheter lumens. If blood cultures remain positive despite 48 hours of appropriate antimicrobial therapy, the catheter should be removed. In addition, removal of the catheter should be considered when there is recurrent bacteremia with the same organism after an appropriate course of therapy.

### Local Catheter Infections

Local catheter infections include exit-site infections, pocket space abscesses or cellulitis, and tunnel infections. Purulence or cellulitis at the catheter exit site, without associated bacteremia, is evidence of a local exit site infection. Warmth, redness, and tenderness are highly suggestive of an infectious etiology, although at times this can be due to inflammation from local irritation or mechanical trauma. Exit site infections can often be managed conservatively with local care with or without systemic antibiotics. Usually these can be managed without catheter removal; however, if *Pseudomonas* is cultured from the site catheter removal may be required.

Pocket space infections present with fluctuance around the subcutaneous catheter hub, often with inflammation or cellulitis of the overlying skin. Tunnel infections are characterized by spreading cellulitis in the subcutaneous tissues along the tunnel tract of long-term i.v. catheters. Unlike exit-site infections, these infections are often associated with serious local morbidity and systemic infection. The pathogens involved are most commonly gram-positive cocci; however, gram-negative bacilli, including *Pseudomonas* as well as *Mycobacterial* sp., are also reported. These infections are best managed by rapid removal of the catheter and treatment with i.v. antibiotics.

### Ear Infections

Children with cancer may develop the same infectious problems as immunocompetent patients. For example, otitis media may follow a viral upper respiratory illness. Clinical findings suggesting an ear infection range from the classic complaints (e.g., ear pain, drainage, fever, irritability) to minimal symptomatology (e.g., slight tympanic erythema) in profoundly neutropenic children. Although the most likely pathogens are identical to those isolated from an immunocompetent host (e.g., *S. pneumoniae*, *H. influenzae*), neutropenic patients are also susceptible to gram-positive or gram-negative bacteria that may have colonized the oropharynx and nasopharynx.[144] Therefore, broad-spectrum antibiotic coverage is necessary unless a specific pathogen has been identified. Patients should receive 10 to 14 days of therapy. Children with anatomic alterations (e.g., radiation damage) of the external or middle ear or eustachian tube are particularly susceptible to recurrent infectious episodes.

Although mastoiditis has become uncommon, immunosuppressed patients, particularly those with an anatomic abnormality of the middle ear, are at risk for the development of mastoiditis. In addition to the usual bacterial pathogens associated with this disorder, individuals with prolonged neutropenia are at risk for fungal mastoiditis, which often requires surgical management for cure. [145] Patients should undergo appropriate evaluation, including computed tomography (CT) scans of the involved area, particularly if they have symptoms or signs (e.g., localized erythema, swelling, tenderness) referable to the mastoid.

### Sinusitis

In the immunocompetent or non-neutropenic child bacterial sinusitis is most commonly caused by *S. pneumoniae, H. influenzae,* and *Moraxella catarrhalis*.[146] *S. aureus*, gram-negative aerobes (including *P. aeruginosa*), and anaerobic bacterial species can also be found, although more with obstruction of the sinuses by tumor (e.g., nasopharyngeal carcinoma, Burkitt's lymphoma, rhabdomyosarcoma) are especially at risk for acute or chronic sinusitis.

Fungal pathogens (e.g., *Aspergillus* sp*., Candida albicans, Mucor*) are particularly worrisome causes of sinusitis in the immunocompromised host. Patients with acute leukemia or other disorders associated with long periods of neutropenia (e.g., aplastic anemia) are especially prone to fungal sinusitis. [144,147] The diagnosis of acute sinusitis is usually suggested by complaints of facial pain, local tenderness, and (assuming a patent outlet and an adequate granulocyte count) purulent nasal drainage. With involvement of the ethmoid sinus, edema of the eyelids and excessive tearing may also be observed. In young children, a nonproductive cough and fetid breath may indicate a sinus infection. In an immunosuppressed patient, however, many of the classic symptoms and signs may be absent, and a high index of suspicion must be maintained, particularly in a persistently febrile, granulocytopenic patient receiving broad-spectrum antibiotics. Any sinus tenderness or even minimal complaints of nasal stuffiness in a neutropenic child should be pursued with conventional radiographs or, preferably, a CT scan and a detailed nasopharyngeal examination, because even subtle findings on physical examination (e.g., minimal crusting on nasal turbinate) may be indicative of an invasive fungal lesion.

In children older than 1 year, radiologic examination of the sinuses is useful for diagnosis. The findings of sinus opacity, an air-fluid interface, or mucosal thickening strongly correlate with acute infection. In patients with chronic sinusitis, radiographic findings are less helpful because of the persistence of abnormalities related to the chronic infection. Serial CT or MRI scans may prove helpful in the immunosuppressed patient with chronic sinus disease because they are more sensitive and more specific than plain radiographs. [148] Specifically, these imaging techniques can facilitate detection of the bony erosion that is common to the indolent fungal pathogens.

Therapy must be tailored to the clinical situation. Acute sinusitis in a non-neutropenic patient is best managed with amoxicillin plus clavulanic acid (Augmentin) or TMP/SMX.[149] For neutropenic patients, broad-spectrum antimicrobial therapy is necessary. Decongestants are an essential adjunct to antimicrobial therapy to provide drainage of the sinuses. If a neutropenic patient with sinusitis does not improve after 72 hours of treatment, aspiration or biopsy of the sinus should be performed. For patients with chronic or recurrent sinusitis, particularly those with a local tumor mass or damage secondary to radiotherapy, an antral window may be necessary to allow adequate drainage.

The diagnosis and treatment of fungal sinusitis in neutropenic patients remain difficult, and the definitive diagnosis depends on histopathologic documentation of tissue invasion. A high level of clinical suspicion is the key to making the diagnosis. Plain radiographs often appear normal even though CT or MRI scans reveal the presence of extensive disease. Patients with prolonged neutropenia in whom fever and mild symptoms of nasal congestion or bleeding develop should undergo such scanning to identify invasive fungal disease. Fungal sinusitis caused by *Aspergillus* or *Rhizopus* sp. can progress to the rhinocerebral syndrome, with invasion through the cribriform plate and into the central nervous system. Early institution of AMB therapy is imperative. Surgical débridement of involved tissue is often required in an effort to remove necrotic and inflammatory material. Even with these aggressive therapeutic maneuvers, a successful outcome depends on the recovery of an adequate granulocyte count.

## INFECTIONS IN THE RESPIRATORY TRACT

Infections of the respiratory tract are among the most common complications in the immunosuppressed cancer patient. Colonization of the upper airway provides a ready source of pathogenic species in direct proximity to the lower respiratory tract. Altered mucosal and humoral immune mechanisms (e.g., subnormal ciliary function, decreased secretory immunoglobulins) provide for less effective clearance of aspirated organisms, and the absence or suboptimal functioning of the phagocytic effector cells (e.g., PMNs, pulmonary macrophages) permits establishment of a local infection and frequently hematogenous dissemination of the organisms.

One of the principal problems in managing pulmonary infiltrates in an immunocompromised host is the number of infectious and noninfectious causes that must be considered, including progression of the underlying malignancy, drug reactions, emboli, and hemorrhage secondary to vascular erosion or severe thrombocytopenia. The most practical approach for the evaluation of an immunocompromised patient with a pulmonary infiltrate is to categorize patients according to the anatomic distribution of the infiltrative lesion (i.e., localized or diffuse) and the granulocyte count (i.e., neutropenic or not). This classification permits rapid evaluation, identification of likely pathogens, and prompt institution of appropriate therapy to optimize the chance for a successful outcome ( Fig. 41-4).

**FIGURE 41-4.** Algorithm for the management of the child with a pulmonary infiltrate.

### Diagnosis of Pneumonia in the Immunocompromised Patient

Pulmonary infections in immunocompromised individuals can progress rapidly to respiratory failure, and therefore, initial evaluation needs to be performed expeditiously. The extent to which a specific etiology for the pulmonic process is investigated will often depend on the clinician's judgment of the immune status of the patient and his or her degree of illness.

The initial evaluation of the child with suspected pneumonia should include a chest radiograph, blood cultures, hematologic indices, pulse oximeter reading (or arterial blood gases in more critical situations), and collection and examination of available culture material. Sputum is usually not available in granulocytopenic patients. Nasopharyngeal aspirates, particularly for viral pathogens, including respiratory syncytial virus, parainfluenza, influenza, and adenovirus can be useful.

Further radiographic evaluation with a CT scan may provide information regarding the pattern and extent of disease that may not be evident on plain film alone. [150] It is important to remember, however, that the radiographic presentation may be extremely variable for any given etiology and that the differential diagnosis of most radiographic findings remains broad.

Flexible bronchoscopy can provide evidence for a specific diagnosis in immunocompromised patients with pneumonia. The yield of bronchoscopy depends on the clinical situation and the extent of prior therapy. [151] Bronchoalveolar lavage (BAL) can yield a specific diagnosis in approximately 80% of cases of PCP, whereas it is significantly less sensitive for fungal infections or bacterial infections in patients who have received prior antibiotic therapy. In a series of 89 bone marrow transplant patients with pulmonary complications of unclear etiology, approximately 50% had a diagnosis made by BAL, including findings of infectious (PCP, bacterial, CMV, RSV) and noninfectious (diffuse alveolar hemorrhage) causes. [152] Transbronchial biopsy may increase the diagnostic yield in some situations; however, this procedure is limited in very small children by the size of the equipment required.

Transthoracic needle biopsy can be useful for the assessment of disease that is focal and in the periphery of the lung parenchyma. Thoracoscopic lung biopsy can provide excellent pathologic samples as well as an ability to visualize superficial lesions, but it is limited in small children by the size of the instruments required. Open lung biopsy remains the gold standard for the diagnosis of pulmonary pathology. Open biopsy allows for sampling of an affected area and can obtain more tissue for pathological analysis. In patients not responding to appropriate empiric antimicrobial therapy with a nondiagnostic workup, an open lung biopsy is often indicated.

### Localized Pulmonary Infiltrate in a Non-Neutropenic Patient

The common causes of a localized pulmonary infiltrate in a non-neutropenic cancer patient are similar to those in an immunocompetent child. Common bacterial, viral, and mycoplasmal organisms are most frequently isolated (Table 41-4), and therapeutic considerations are similar to those for an immunocompetent child. Non-neutropenic patients who are receiving immunosuppressive therapy (including corticosteroids) are also at risk for unusual pathogens, including *Mycobacterium tuberculosis*, atypical mycobacteria, *Nocardia asteroides*, *Legionella* sp., *Aspergillus* sp., *C. neoformans*, *Chlamydia* sp., and certain endemic mycoses such as *H. capsulatum* and *C. immitis*.

**TABLE 41-4. MODIFICATIONS OF INITIAL ANTIMICROBIAL REGIMEN FOR FEBRILE NEUTROPENIC CANCER PATIENTS**

Specific mycobacterial identification is especially important to distinguish atypical isolates (particularly *Mycobacterium kansasii* or *Mycobacterium avium-intracellulare*) from true *M. tuberculosis*. Children with documented drug-sensitive *M. tuberculosis* should receive 9 to 12 months of therapy with at least two effective antituberculosis agents (e.g., isoniazid and rifampin). However, it is critical to consider the possibility of multidrug-resistant strains of tuberculosis, particularly in patients who live in areas where those strains are endemic. [153]

A less common but nevertheless important pathogen to consider as the cause of localized pneumonia in an immunosuppressed child is *Legionella*. *Legionella* sp. are commonly found in water sources and may be found in the hospital environment, leading to nosocomial acquisition. *Legionella* pneumonia in immunosuppressed pediatric patients is heralded by abrupt onset of nonspecific symptoms such as fever, malaise, anorexia, lethargy, and headache. A nonproductive cough develops in most patients; chest pain, dyspnea, diarrhea, and neurologic symptoms may be prominent features. [154,155] A lobar, patchy, alveolar infiltrate is most common, although diffuse infiltrates may occur. [156] A definitive diagnosis of *Legionella* pneumonia is made by culture of the organism from respiratory secretions. The urinary antigen test is specific only for *Legionella pneumophila* type 1, which causes 80% of cases. [157] Other rapid tests for diagnosis of *Legionella* infection (direct fluorescent antibody stain, DNA probe) lack the sensitivity and specificity of culture. Erythromycin (40 to 50 mg/kg per day, maximum of 4 g) given for 3 weeks is the treatment of choice, with addition of rifampin for seriously ill patients.

*Nocardia*, particularly *N. asteroides*, may also present as a localized pulmonary infiltrate, although miliary and microcavitary patterns have been described in cancer patients. [158] Approximately 30% of patients with a pulmonary infection caused by *N. asteroides* also have cutaneous infection and central nervous system involvement (usually brain abscesses). Diagnosis depends on positive cultures or histopathologic demonstration of tissue invasion by the organisms. TMP/SMX (15 mg per kg per day TMP) for 6 months is the most practical therapy. The mortality rate of disseminated disease remains at approximately 30%.

Noninfectious causes for a localized pulmonary infiltrate in a non-neutropenic patient should also be considered, including progression of the underlying malignancy, an atelectatic segment of lung (potentially caused by compromise of the airway by adjacent tumor), pulmonary hemorrhage, and a localized reaction to a chemotherapeutic agent (particularly methotrexate, cyclophosphamide, or bleomycin), although drug reactions are more commonly manifested as diffuse interstitial

pulmonary processes.

**Localized Pulmonary Infiltrate in a Neutropenic Patient**

An array of opportunistic pathogens must be considered in the neutropenic patient with a localized infiltrate, including those noted in the non-neutropenic patient. Any gram-positive or gram-negative organism as well as a variety of fungal, parasitic, and viral pathogens can be responsible ( Table 41-4). Bacterial pathogens predominate in patients with neutropenia lasting less than 14 days. Patients with longer periods of neutropenia and those in certain clinical settings (e.g., allogeneic bone marrow transplantation) are more prone to develop a fungal (e.g., *Aspergillus*) or viral (e.g., CMV) infection. Unless the clinical presentation suggests otherwise, it is appropriate to initiate a 48- to 72-hour trial of broad-spectrum antibiotics before proceeding to an invasive diagnostic procedure. If the patient has stabilized or improved by 72 hours, a 10- to 14-day course of treatment is necessary. If the patient has not stabilized or improved, a BAL or open lung biopsy should be performed.

The most frequently encountered cause of a localized pulmonary infiltrate in the patient with protracted neutropenia is a fungal pneumonia, particularly if the patient already is receiving broad-spectrum antibiotics.[159,160] *Aspergillus* sp. are most often responsible, although *P. boydii*, *Fusarium* sp., *Trichosporon beigelii*, and the Zygomycetes (e.g., *Rhizopus* sp.) may also play a role.[161,162] Occasionally, *Candida* sp. (especially *C. albicans* and *C. tropicalis*) may cause hematogenous or primary pulmonary candidiasis.[163] Other fungi, including *H. capsulatum*, *C. immitis*, and *C. neoformans*, can also cause focal pneumonia in neutropenic patients receiving corticosteroids, although infections with these organisms are more commonly manifested as a diffuse or nodular pulmonary pattern.[164] The clinical, laboratory, and radiographic features of fungal pathogens are indistinguishable from those of other pulmonary processes, and definitive diagnosis depends on microbiologic or histopathologic confirmation in specimens obtained from transbronchial or open lung biopsy.

The incidence of pulmonary infections caused by *Aspergillus* sp. has increased in recent years and has been observed in clusters in certain hospitals.[162,165,166] The most common scenario is that of a profoundly and persistently neutropenic patient who develops a localized, progressive pulmonary infiltrate while receiving broad-spectrum antibiotics. Infections caused by *Aspergillus fumigatus* and *Aspergillus flavus* are the most common, presumably initiated by inhalation of airborne conidia. Because of the tendency for *Aspergillus* sp. to invade blood vessels, a necrotizing bronchopneumonia is characteristic, with the possibility of life-threatening hemoptysis. Disseminated aspergillosis occurs in approximately 30% of the cases, with involvement of the central nervous system, liver, kidneys, skin, and spleen. Despite this propensity for widespread disease, blood cultures are almost never positive.

Diagnosis of aspergillosis by noninvasive measures remains suboptimal, although positive culture of sputum or BAL specimens from a patient with long-term fever and neutropenia and a progressive infiltrate is highly associated with *Aspergillus* pneumonia. A definitive diagnosis still requires histologic confirmation or a positive BAL culture obtained in a clinically relevant setting.

Control of *Aspergillus* pneumonia requires an early diagnosis and prompt intervention. Therapy is provided by AMB at 1.0 to 1.5 mg per kg per day. Invasive pulmonary aspergillosis can develop in patients who are receiving empiric AMB at 0.5 to 0.6 mg per kg per day or lipid formulation of AMB for persistent fever and prolonged neutropenia. Higher dosages of AMB (1.0 to 1.5 mg per kg per day) or lipid formulation of AMB (greater than or equal to 5 mg per kg per day) are required to contain aspergillosis in this setting. Even with potent pharmacologic intervention, the most important prognosticator of a successful outcome is recovery from neutropenia. Subsequent cycles of chemotherapy-induced neutropenia may result in recrudescence of *Aspergillus* pneumonia unless antifungal therapy is continued during these periods of immunosuppression.

Lipid formulations of AMB offer the potential of treating sinopulmonary aspergillosis with higher dosages and less nephrotoxicity. These compounds may be particularly important in patients receiving concomitant nephrotoxic agents, such as aminoglycosides, cyclosporin, and foscarnet.

Other filamentous fungi, such as *P. boydii, Fusarium* sp., dematiaceous molds, and the Zygomycetes, especially *Rhizopus* sp., can cause pulmonary infiltrates that are similar to those associated with *Aspergillus*.[167,168] These organisms may also cause sinus infections and the rhinocerebral syndrome. Diagnosis requires documentation of tissue invasion. Therapy consists of AMB (1.0 mg per kg per day) and, if appropriate, aggressive surgical débridement. Nevertheless, treatment results remain poor unless granulocyte recovery ensues.

**Diffuse Pulmonary Infiltrates in a Cancer Patient**

Diffuse pulmonary infiltrates can be caused by bacterial, viral, fungal, or protozoal pathogens; the probability of having a specific pathogen is influenced by whether the patient is neutropenic (Table 41-4). A non-neutropenic patient with a diffuse pulmonary infiltrate is unlikely to have a bacterial or fungal process; however, both groups of patients are at risk for severe infection from *Pneumocystis pneumoniae* and various viral pathogens.

One of the most commonly encountered infections in this setting is PCP.[121] This infection is thought to result from a reactivation of latent cysts, because almost all normal children possess detectable antibody to the organism. However, patient-to-patient transmission has been suggested by reports of nosocomial clusters of cases.[169] It is also possible that certain chemotherapeutic regimens predispose patients to interstitial infiltrates caused by *P. carinii*.

Patients with *Pneumocystis* pneumonia most commonly present with fever, a nonproductive cough, tachypnea, and hypoxemia. The time course of the symptoms ranges from a chronic, indolent course (characteristic of patients with AIDS) to an acute, fulminant presentation, more common in pediatric oncology patients.[170] Rales are not usually detectable on auscultation. Radiographic examination usually reveals bilateral diffuse interstitial infiltrates, often originating at the hilum and extending peripherally. Rarely, the chest radiograph is atypical, ranging from normal to a lobar or nodular infiltrate. Pleural effusions are rare.

Diagnosis of *Pneumocystis* pneumonia requires demonstration of cysts or trophozoites in pulmonary material from patients with a clinically compatible course. In patients with AIDS (including children), positive specimens may readily be obtained from induced sputum samples stained with toluidine blue O, a modified Giemsa stain, or with monoclonal antibodies to human *Pneumocystis* organisms. The sensitivity of sputum examination in an HIV-infected patient population has increased from initial reports of 55% with the routine stains to as high as 92% with monoclonal antibody stains detected by indirect immunofluorescence. A specificity of almost 100% has been reported from experienced laboratories.[171,172] In cancer patients, *Pneumocystis* organisms may not be as abundant as in patients with AIDS.[173] The diagnosis of *Pneumocystis* pneumonia in an immunosuppressed cancer patient can sometimes be made by monoclonal staining techniques on induced sputum, and this should be the first diagnostic approach whenever possible.[170] The demonstration of cysts, however, may require BAL or, in some cases, open lung biopsy.

In clinical situations in which the likelihood of *Pneumocystis* pneumonia is great, examination of induced sputum is a reasonable first step in older children. If the specimen is not attainable or if there is a negative result, it is best to continue on to a BAL while initiating an empiric course of TMP/SMX. A response to TMP/SMX may not be apparent for 4 to 5 days, although stabilization or slight improvement in alveolar air exchange usually occurs within 72 to 96 hours. Patients who do not respond should be given pentamidine at a daily dose of 4 mg per kg i.v. Pentamidine has been associated with myriad toxicities, including metabolic and hematologic abnormalities, pancreatitis, hypotension, and nausea and vomiting. Evidence from the adult AIDS population suggests that atovaquone or dapsone plus TMP may be acceptable alternatives to TMP/SMX or pentamidine in patients with mild or moderate disease.[174,175]

The early use of adjunctive steroids has been shown to improve the outcome in adult patients with AIDS with moderate or severe *Pneumocystis* pneumonia, defined by a room air arterial partial pressure of oxygen equal to 70 mm Hg or less, or an alveolar-arterial gradient greater than 35 mm Hg on presentation. Decreases in respiratory failure and death rates were observed among patients who received steroids with standard antiprotozoal therapies, compared with those who did not receive steroids.[176,177] Based on this information, the practice of initiating a short course of steroids in children with moderate or severe *Pneumocystis* pneumonia has been adopted by many pediatricians, although the optimal dose and duration of steroid therapy in children have not been defined. The recommendation for adults is for 40 mg prednisone (or the equivalent steroid) b.i.d. for the first 5 days of treatment, 40 mg once daily for the next 5 days, and then 20 mg once daily for 11 days, for a total treatment course of 21 days. A roughly estimated equivalent for children is 1 mg per kg b.i.d. for the first 5 days, 1 mg per kg daily for the next 5 days, and 0.5 mg per kg daily for the remainder of a 14- to 21-day course of therapy.

*Mycoplasma pneumoniae* can cause diffuse pulmonary infiltrates and severe disease in immunocompromised children. Evaluation of BAL fluid by polymerase chain reaction (PCR) for mycoplasma can be diagnostic. Often an empiric course of azithromycin is warranted until definitive diagnosis can be made.

Viruses are an important cause of diffuse interstitial infiltrate in non-neutropenic patients. Before routine prophylaxis and strategies for “preemptive therapy” (see below) CMV pneumonitis was a common cause of viral pneumonia after allogeneic bone marrow transplantation. CMV pneumonitis has also been seen in a very small percentage of patients after autologous bone marrow transplantation.[178] The most frequent causes of CMV infection and subsequent disease are reactivation of latent

virus in seropositive patients and acquisition of CMV from donor marrow in seronegative patients. CMV pneumonitis most often occurs between day 30 and day 100 after allogeneic bone marrow transplantation, coinciding with the period of highest risk for the development of acute graft-versus-host disease. Radiographically, CMV pneumonitis is characterized by diffuse bilateral linear or nodular infiltrates, although it is occasionally represented by a unilateral consolidation or a single nodule. [179]

The use of CMV antigenemia assays as well as CMV PCR has allowed for the rapid diagnosis of CMV infection. Routine screening during the period of highest risk, for example with twice weekly CMV antigen assays, has proved a highly effective strategy to prevent invasive disease. Detection of CMV or antigen in blood buffy coat post–bone marrow transplantation is a sign of CMV reactivation that is highly predictive of impending invasive disease. Such findings should prompt the institution of a treatment course with ganciclovir. Ganciclovir therapy has significant toxicity, primarily causing granulocytopenia, which much be monitored carefully. Alternative therapy can be accomplished with foscarnet.

The diagnosis of CMV pneumonia can be made by isolating CMV from bronchoalveolar fluid in the proper clinical setting and demonstrating either CMV antigen or nucleic acid in alveolar macrophages or compatible tissue pathology. Until recently, CMV pneumonitis has been associated with an extremely high mortality (greater than 85%) despite the use of a variety of antiviral and immunotherapeutic agents. Combined therapy with ganciclovir and i.v. immune globulin (pooled or CMV hyperimmune) has markedly improved survival to more than 50% in allogeneic bone marrow transplantation patients with CMV pneumonitis. [180,181] Despite the limited enrollment and uncontrolled nature of these trials, the dramatic results have led to an acceptance of the ganciclovir and immune globulin combination as a standard of care for patients with CMV pneumonitis. Whether i.v. immunoglobulin is a useful adjunct to ganciclovir for CMV pneumonitis or even colitis in other types of patients is less clear.

Other herpesviruses, VZV and HSV, can also cause diffuse pneumonitis. These viruses, however, rarely cause isolated pulmonary disease but instead are associated with visceral dissemination from dermatomal or cutaneous disease.

RSV can cause severe lower respiratory tract disease with a high mortality rate, especially in patients undergoing therapy for AML or bone marrow transplantation. [108,109] RSV usually can be diagnosed rapidly using a direct immunofluorescent antibody stain performed on a nasal wash specimen.

Although no randomized controlled studies have addressed the utility of ribavirin in immunocompromised cancer patients, it may be appropriate to extrapolate from existing data in other pediatric populations that suggests that there may be a benefit of this therapy. [103,104,106] There is anecdotal evidence from several small case series reports that adult bone marrow transplantation and acute leukemia patients with lower tract RSV respond favorably to ribavirin aerosol in addition to therapy with i.v. immunoglobulin or RSV immune globulin. [109,114] Survival appeared to be improved particularly if the drug was administered early in the course of infection.

Adenovirus, parainfluenza virus, influenza, and human herpesvirus type 6 have been described as causes of interstitial pneumonitis in pediatric cancer patients. All of these entities cause diffuse interstitial pneumonitis and are also associated with a high rate of mortality. [182,183]

**Infections of the Gastrointestinal Tract**

The GI tract is frequently a source of infection in the neutropenic patient. The GI mucosa is in direct contact with the external environment. Normally, it acts as a mechanical barrier, but it can be disrupted by tumor invasion or by damage from chemotherapy or radiotherapy. The mucosal ulceration induced by these treatments offers a potential site for bacterial, fungal, and viral colonization, invasion, and infection. The normal microbial balance of the GI tract is also altered by serious illness, mechanical factors (e.g., surgery, altered motility), hospital exposures, and antimicrobial therapy, further contributing to colonization and potential infection.

**Infections of the Oral Cavity**

The most common mucosal infection encountered in the immunosuppressed cancer patient is thrush, a superficial oral infection caused by *C. albicans*. The lesions usually appear as whitish plaques with slightly raised indurated borders. This infection is easily controlled in most cases by topical antifungal agents, such as clotrimazole troches. If there is no response to topical therapy, fluconazole at doses of 50 to 100 mg per day has proved highly effective for the treatment of oropharyngeal candidiasis. [184] Despite their relatively benign appearance and ease of control, superficial fungal infections may serve as a nidus for systemic dissemination and contribute to poor nutrition.

HSV is the most common viral pathogen isolated from mucosal lesions. Clinically, the lesions usually appear as clear vesicular eruptions, frequently in clusters or “crops” on an erythematous base, either periorally or intraorally. However, intraoral lesions may be nondescript, are often ulcerative, and can be confused with the stomatotoxicity usually attributed to chemotherapy. Diagnosis may be confirmed by rapid shell vial culture (requiring 24 to 48 hours) or may be made presumptively by the identification of multinucleated giant cells (positive Tzanck preparation) or by a positive fluorescent-antibody reaction. Unlike the self-limited and relatively innocuous presentation in an immunocompetent host, herpetic stomatitis may be a serious complication in an immunosuppressed patient. The severity of the local tissue involvement, inflammation, and eschar formation has deleterious consequences, causing discomfort and also serving as a nidus for bacterial superinfections. Acyclovir (750 mg per $m^2$ per day divided every 8 hours) is the preferred treatment because of its infrequent toxicity, ease of administration, and documented efficacy in reducing the duration of viral shedding and shortening the time to healing. [185] HSV-seropositive patients undergoing bone marrow transplantation or induction regimens for acute leukemia should receive prophylactic acyclovir, orally or parenterally, to prevent HSV reactivations.

If the patient does not have specific local or systemic indications of infection, management of mucosal ulceration should be directed toward symptom management. There is no evidence that maintenance of oral hygiene reduces the incidence or severity of oral mucositis induced by chemotherapy, although it is probably beneficial with regard to reduction of infectious complications.

Periodontal disease (including gingivitis and periodontitis) is especially problematic among adults, and the incidence of periodontal disease in the general population increases with age. However, periodontal disease may be found among pediatric cancer patients and is related to the adverse effects of the antineoplastic therapy on the host defense mechanisms normally operative in the oral mucosa. Gingivitis is an inflammation of the superficial structures of the mucosal epithelium, and periodontitis describes an involvement of the supportive structures of the teeth. A prospective study of 38 febrile patients receiving therapy for acute myeloblastic leukemia revealed a 32% incidence of oral infections. [186] The periodontium was the most common site, and cultures of infected sites revealed mixed aerobic and anaerobic flora. [187] The presence of marginal or necrotizing gingivitis, characterized by an erythematous periapical gingiva, is caused by anaerobes and should be treated with specific anti-anaerobic agents such as clindamycin, metronidazole, or imipenem.

**Esophagitis**

Clinically significant esophagitis may result from infectious or noninfectious causes. A syndrome clinically identical to infectious esophagitis occurs in patients who have received extensive chest wall or mediastinal irradiation, or it may result from the severe mucosal toxicity that is associated with certain chemotherapeutic regimens.

Infectious esophagitis most commonly occurs among neutropenic patients. Patients most often present with subacute onset of retrosternal burning chest pain and odynophagia. Fungal, viral, and bacterial organisms can all cause an infectious esophagitis in the immunocompromised host. [188,189]

The occurrence of infectious esophagitis in a non-neutropenic patient is rare. In non-neutropenic patients, esophagitis is most commonly caused by chemical irritation of the distal esophagus by refluxed gastric contents, as may be associated with chemotherapy-induced emesis. These patients are best managed with judicious use of antacids, histamine antagonists, or omeprazole. If the non-neutropenic patient has persistent esophageal discomfort, esophagoscopy with brushings for culture and biopsy should be performed. In non-neutropenic patients with AIDS, herpetic or *Candida* esophagitis is the most common infection.

Chemotherapy-induced mucositis, neutropenia, mediastinal radiation, and gastroesophageal reflux are important risk factors for esophageal candidiasis. Concomitant infections caused by HSV, CMV, and bacteria may coincide with or precede esophageal candidiasis. The absence of oral lesions cannot be used to discount the presence of esophageal candidiasis. Esophagoscopy with mucosal biopsy is the most definitive method for establishing a diagnosis, but it may not be feasible, practical, or safe in many children and is not recommended in those who are neutropenic. Accordingly, an empiric approach is often warranted in children with suspected esophageal candidiasis. Initial therapy with oral fluconazole is often effective; however, failure to symptomatically respond promptly is an indication for empiric AMB. Furthermore, the resolution of symptoms does not necessarily signify the eradication of esophageal candidiasis in granulocytopenic patients, and

therapy should continue until the resolution of the neutropenia. Persistent symptoms may indicate the presence of another infectious process, such as HSV, CMV, or bacterial esophagitis. If the patient has persistent symptoms after 48 hours of i.v. AMB, an empiric course of acyclovir (750 mg per $m^2$ per day, given at 8-hour intervals) is reasonable, because the second most likely pathogen (or co-pathogen) is HSV. If the patient responds, acyclovir should be given for 7 days. If symptoms do not improve, esophagoscopy and biopsy should be pursued if feasible.

**Intra-Abdominal Infections**

The clinical presentation of even common intra-abdominal processes such as appendicitis or infectious diarrheal syndromes can be altered by granulocytopenia and compounded by complications of cancer or its treatment. For example, obstructive lesions may be caused by primary or metastatic cancer; cholangitis or a conjugated hyperbilirubinemia may be caused by extrahepatic biliary obstruction by tumor; and chronic abdominal pain or diarrheal syndromes may be secondary to bowel wall infiltration by malignant disease or infection.

Intra-abdominal complaints must be expeditiously evaluated with a thorough physical examination, including a judiciously performed rectal examination. Repetitive rectal examination must not be performed in the neutropenic patient, because bacteremia and local infection may result. Appropriate studies include routine hematologic and serum chemistry values, amylase, total and direct bilirubin, and flat and upright abdominal radiographs. Additional diagnostic procedures such as abdominal or pelvic ultrasound, CT, or MRI should be pursued in the appropriate settings. Invasive diagnostic or radiographic procedures such as barium enema and endoscopy should be avoided in the neutropenic patient.

Typhlitis is a necrotizing infection of the cecum restricted almost entirely to cancer patients. Typhlitis most commonly occurs in association with prolonged granulocytopenia in patients with acute leukemia, although any granulocytopenic patient is at risk. Patients most often present with subacute or acute onset of right lower quadrant abdominal pain, which frequently becomes generalized over several hours, with fever, diarrhea, and prostration. The etiologic agents responsible for typhlitis include anaerobes and gram-negative bacillary organisms, especially *P. aeruginosa*. *Clostridium* sp., including *C. difficile*, may also be responsible. Optimal management initially involves supportive care, including nasogastric suction, adequate fluid replacement, and adjustment of antimicrobial therapy to cover resistant gram-negative and anaerobic species. The most sensitive diagnostic radiographic technique is CT or MRI, which should be performed in all neutropenic patients with right lower quadrant pain for prompt diagnosis.[190] Aggressive surgical intervention to resect necrotic bowel may be beneficial for a subset of patients, and the timing of such intervention is critical. Criteria that have been proposed for sending a patient to surgery are persistent GI bleeding after resolution of neutropenia; thrombocytopenia and clotting abnormalities; evidence of free intraperitoneal perforation; clinical deterioration requiring support with vasopressors or large volumes of fluid, suggesting uncontrolled sepsis; and development of symptoms of an intra-abdominal process, in the absence of neutropenia, which would normally require surgery.[191,192]

**Other Abdominal Infections**

An infrequently encountered clinical syndrome is peritonitis and bacteremia caused by *Clostridium*. Patients with clostridial peritonitis classically have a fulminant clinical course with fever, tachycardia, abdominal wall ecchymoses and crepitance, and significant hemolysis.[193] *Clostridium perfringens* and *Clostridium septicum* are the organisms most frequently isolated. Serious infections with *Clostridium* (especially *C. septicum*) can occur in the absence of fever and should be considered in the afebrile neutropenic patient with abdominal pain. A less fulminant bacteremic syndrome caused by *Clostridium tertium* has also been described in granulocytopenic children with acute leukemia who received broad-spectrum antimicrobial therapy for long periods.[194] Most *Clostridium* isolates are sensitive to the penicillins, cephalosporins, and clindamycin, with the exception of *C. tertium*, which requires vancomycin therapy.

Colitis has long been associated with the administration of clindamycin, ampicillin, and broad-spectrum b-lactam antibiotics. *C. difficile*, a normal component of intestinal flora in approximately 3% of healthy adults, has been isolated as the causative agent in most cases.[195] The symptomatic disease is related to *C. difficile* colonization of the gut after some perturbation of the normal gut flora, followed by overgrowth and toxin production by the organism.[196] In cancer patients, antineoplastic agents and antibiotics contribute to the alteration of intestinal flora and increase the risk of *C. difficile* colitis. Patients classically present with acute generalized abdominal pain, fever, leukocytosis, and watery or mucoid foul-smelling diarrhea. A high index of suspicion is necessary because of the occurrence of similar abdominal symptoms in cancer patients receiving chemotherapy or periabdominal radiation. Cancer patients with diarrhea with or without fever and abdominal pain should be evaluated with routine stool cultures and a *C. difficile* toxin assay. Toxin production, not just a culture positive for *C. difficile*, is necessary for diagnosis, because as many as 42% of hospitalized patients receiving antibiotics are culture positive but not toxin positive.[197]

Treatment of documented *C. difficile*–associated colitis is accomplished with either oral vancomycin or metronidazole. The use of vancomycin as first-line therapy is discouraged because of concern about emergence of resistant gram-positive organisms. There is a 10% to 20% rate of relapse, although most patients respond to a second course with the same or alternative therapy. Some patients have repetitive episodes of *C. difficile* diarrhea with each cycle of chemotherapy. *C. difficile* may be nosocomially transmitted, and patients who are culture and toxin positive should be placed on enteric precautions.

An infrequently encountered clinical problem is a hyperinfection syndrome caused by the intestinal nematode *Strongyloides stercoralis*.[198] The clinical syndrome of fever, nausea, vomiting, diarrhea, and abdominal pain is caused by invasion and ulceration of the GI mucosa by the filariform larvae. Chemotherapy is thought to promote the maturation of these larvae from a quiescent rhabditiform stage. Polymicrobial sepsis may accompany the intestinal invasion, presumably as a result of the ulcerated mucosa. Overwhelming pulmonary and meningeal involvement has been described in immunocompromised patients. Diagnosis requires demonstration of the larvae in feces or duodenal fluid and should be sought in patients who have resided in subtropical climates or endemic regions. Treatment of asymptomatic infestation is accomplished with thiabendazole (25 mg per kg b.i.d. for 2 days) or ivermectin, 200 µg per kg. Ivermectin has evolved as the preferred drug with efficacy comparable to that of thiabendazole but with less toxicity. Immunocompromised patients with the hyperinfection syndrome should be treated for 2 to 3 weeks, although the mortality rate remains high despite such long-term treatment.[199] Due to its long half-life, ivermectin may be used on days 1, 2, 13, and 14 of a 2-week treatment cycle.

**Hepatic Infections**

Hepatitis may be caused by a variety of infectious agents, including those that infect the liver primarily (e.g., hepatitis A, B, C, and the delta agent) and those that infect secondarily (e.g., HSV, CMV, Epstein-Barr virus, coxsackie B virus, adenovirus, toxoplasmosis, *C. albicans*). In addition, many chemotherapeutic agents and other medications that immunocompromised cancer patients may be receiving are associated with noninfectious hepatitis. In addition to the morbidity (e.g., fever, nausea, emesis, arthritis, arthralgia) and deaths directly attributable to hepatitis, significant alteration in hepatic function can affect the pharmacokinetics of antineoplastic agents and other medications and should be taken into consideration when dosing drugs.

Hepatitis C virus (HCV) is now well-recognized as the predominant cause of classic transfusion-associated non-A, non-B hepatitis.[200,201] More recently it has been recognized as a significant issue for long-term survivors of childhood cancer. In one study of cancer patients who had been transfused between 1961 and 1992, 6.6% were found to be infected with HCV.[202] At the time of infection most cases are subclinical but can be characterized by fatigue and hepatomegaly with elevated or fluctuating levels of aminotransferases. Incubation is approximately 8 weeks, with the onset of symptoms, if any, typically occurring 5 to 12 weeks after exposure. Approximately 85% of patients infected with HCV develop chronic infection.[111] Of those with chronic hepatitis, 20% to 25% develop cirrhosis, and 2% to 9% die due to complications of cirrhosis or hepatocellular carcinoma.

Persistent antihepatitis C antibody is usually but not always found in patients with chronic disease, whereas those with the acute, self-limited illness may have only a transient rise in antibody titers. The development of antibody after hepatitis C exposure is usually delayed, with a mean interval of approximately 20 weeks and occasionally much longer. Late serologic testing is therefore essential for the diagnosis of chronic hepatitis C.[203] All patients positive for HCV antibodies by enzyme immunoassay or by recombinant immunoblot assay should be tested for HCV RNA by PCR. In addition, the viral genotype should be determined, as this information may impact therapy.[110]

Some patients with chronic HCV infection can be effectively treated with a combination of interferon and ribavirin. The decision of who and when to treat is based on a number of factors, including severity of disease, concurrent comorbidity and preexisting contraindications to ribavirin or interferon. There have been two large randomized, controlled trials of combination therapy in patients with documented HCV infection and persistently elevated transaminases, and without significant coexisting medical conditions or decompensated hepatic cirrhosis. In both studies patients had improved outcomes with combined therapy compared to monotherapy.[204,205] In these studies the overall rate of sustained response for the combination regimen was 64% to 69% in patients with genotype 2 and 3 and 28%

for those with genotype 1. The combination regimen has significant side effects that required medication discontinuation in 8% to 19%.

Hepatitis B may result in acute or chronic infections, including chronic active hepatitis, chronic persistent hepatitis, and asymptomatic carrier states. The incidence of hepatitis B was previously as high as 10% to 20% among cancer patients, but with the introduction of effective prophylactic measures, particularly widespread use of hepatitis B vaccine and efficient screening methods to detect infected donors, the number of patients affected has fallen dramatically. Immunosuppressive therapy may increase the likelihood of development of a chronic carrier state among patients infected with hepatitis B, making it important to know the patient's hepatitis status before initiating antineoplastic therapy. In addition, an acute hepatic failure syndrome may result from acute infection in an immunosuppressed patient. [206,207] Lamivudine is licensed for the therapy of hepatitis B in adults, but there are no data available for use in children.

The delta agent, an incomplete RNA virus, requires prior infection or coinfection with the hepatitis B virus to manifest clinically. Therefore, hepatitis caused by the delta agent occurs only in three circumstances: as a superimposed infection in a patient with active hepatitis B, as an acute hepatitis in a chronic hepatitis B carrier, and as a chronic infection in a chronic hepatitis B carrier. Coinfection with hepatitis B and delta virus can produce a more fulminant or severe hepatitis than can infection with hepatitis B alone. In the United States, the incidence of delta virus is low and will presumably decrease further as hepatitis B incidence diminishes with widespread vaccination efforts. [208,209]

Several viruses may involve the liver secondarily as part of a more widespread systemic infection. The Epstein-Barr virus, CMV, HSV, VZV, rubella, rubeola, mumps, adenovirus, and coxsackie B virus have been associated with hepatic enzyme elevations. The hepatic dysfunction attending these secondary infections is usually self-limited and less severe than that associated with primary viral hepatitis. However, fulminant hepatic necrosis, coma, and death have been described with several of these agents (especially HSV, CMV, and VZV) in the immunocompromised host. Patients with acute hepatitis caused by HSV or VZV should receive acyclovir, and those with CMV hepatitis may require ganciclovir or foscarnet.

All cancer patients with clinical or biochemical evidence of hepatitis should undergo a serologic evaluation to attempt to characterize the etiologic agent. Serum tests for hepatitis B surface antigen, antibodies to hepatitis A (IgM and IgG), and hepatitis B core antigen (IgM and IgG) can identify patients with hepatitis A or B. Repeated testing for the development of antibody to hepatitis C over a period of weeks to months may be required to diagnose this infection, in addition to evaluation by PCR for HCV RNA. Patients with a negative antibody screen for all of these viruses can have hepatitis caused by an agent other than the classic hepatitis viruses or a noninfectious cause. In addition to viral infection, hepatic enzyme elevation or hyperbilirubinemia can occur with bacterial sepsis, fungal infection of the liver (especially *Candida* or *Aspergillus*), or toxoplasmosis.

**Hepatic Candidiasis**

A syndrome referred to as *hepatic candidiasis* occurs in patients recovering from a long period (usually more than 7 days) of neutropenia. [210,211] It is characterized by the presence of bull's-eye lesions in the liver on ultrasound or CT scan ( Fig. 41-5). These lesions are not apparent in patients who are neutropenic but rather become recognizable at the time of neutrophil recovery. Patients have persistent fever after recovery from an episode of neutropenia, frequently with right upper quadrant discomfort, nausea, and increased serum alkaline phosphatase. The lesions are granulomas and consist of an inner core of necrosis (in which the yeast and pseudohyphae can be found) surrounded by a ring of inflammatory cells and an outer ring of fibrosis. These imaged lesions change over time and with treatment and resolution may become calcified, an important end point of therapy.

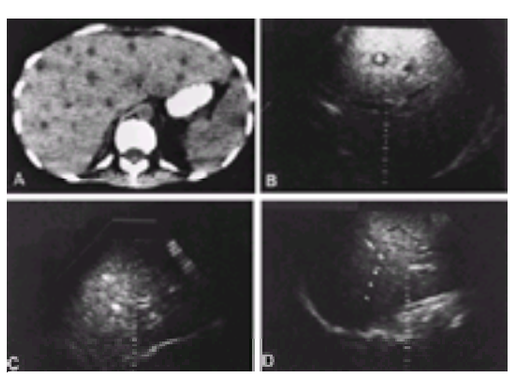



**FIGURE 41-5. A:** Computed tomography scan of the liver shows numerous rounded areas of decreased attenuation compatible with the diagnosis of hepatic candidiasis. This is a nonspecific finding. **B:** Ultrasound examination in the same patient shows the typical bull's-eye lesion of candidiasis characterized by a central echogenic nidus surrounded by a radiolucent halo. This is seen early in the natural history of the disease. **C:** The radiolucent halo is now less obvious than in **(B)**. This illustrates the variable appearances of candidal abscesses on ultrasound studies at different times in the same patient. **D:** Later in the course of the disease, the microabscesses become denser (*arrow*). Note the acoustical shadow posterior to the lesion, caused by attenuation of the sound beam ( *arrowheads*). (From Thaler M, Bader J, O'Leary T, Pizzo PA. Hepatic candidiasis in immunocompromised patients. Ann Intern Med 1988;108:88–100, with permission.)

The pathogenesis of hepatic candidiasis is one of chronic disseminated candidiasis—that is, the liver as well as the spleen, kidneys, lungs, brain, eyes, and other tissues may be infected by antecedent candidemia. Blood cultures may be negative before the diagnosis of hepatic candidiasis and are almost invariably negative on recovery from neutropenia. The diagnosis is based on a high index of suspicion and should be confirmed with liver biopsy. Hepatic tissue may be obtained by a small midline open incision in children and by laparoscopy in adults. These procedures in our experience are well tolerated in patients with hepatic candidiasis. Splenic lesions may be the only apparent abscesses on CT scan. On laparoscopy or biopsy, however, the liver in these cases is usually found to be infected by small lesions below the threshold of CT scan detection. MRI scans with gadolinium contrast may reveal lesions that are not otherwise visible on CT scans. The detection of the *Candida* enolase antigen and D-arabinitol in blood has been used as an adjunctive tool for diagnosing invasive candidiasis, including hepatic candidiasis, with encouraging results. [212,213]

Hepatic candidiasis poses a therapeutic challenge. Long courses of treatment are necessary, with the average duration of AMB administration approximately 6 to 12 months. The addition of 5-FC may further enhance antifungal activity if the kidney or CNS is involved. Current experience with lipid formulation of AMB indicates that it is effective in the treatment of hepatosplenic candidiasis, with curative total doses of drug being delivered in a much shorter time and with fewer side effects than those that have been seen with conventional AMB. [87] Experimental data and several encouraging reports suggest that fluconazole has significant efficacy in the treatment of hepatosplenic candidiasis in patients in whom AMB has not controlled the infection or who have had serious AMB-related toxicities. [214,215] and [216] Failures of fluconazole in this setting have also been described, however, suggesting that caution should be exercised until definitive clinical trials are performed. A reasonable approach is to treat children initially with AMB and 5-FC and, after the patient is afebrile or has response of lesions to change therapy to fluconazole at 12 mg per kg in two divided doses. AMB lipid complex would be indicated in children refractory to or intolerant of AMB or fluconazole.

Whether patients with hepatosplenic candidiasis should continue to receive antineoplastic therapy, which may cause neutropenia with the risk for progressive hepatosplenic involvement or breakthrough fungemia, can be a major dilemma. We have found that this infection in patients with cancer can be treated successfully under careful observation through repeated courses of chemotherapy-induced neutropenia without progression of hepatosplenic candidiasis or breakthrough fungemia. [217]

**Perianal Cellulitis**

The overall incidence of perianal cellulitis has decreased in recent years, presumably because of the early use of empiric antibiotics when granulocytopenic patients become febrile. Nonetheless, the risk for perianal cellulitis remains, especially for patients in the high-risk category, those with chronic (more than days) and profound (less than 100 cells per $mm^3$) granulocytopenia. Predisposing factors include perirectal mucositis caused by chemotherapy or localized radiotherapy, hemorrhoids, anal fissures, and any type of rectal manipulation (e.g., barium enema, anoscopy, sigmoidoscopy). Accordingly, constipation should be avoided, because passage of hard stool promotes the formation of anal fissures and increases the risk of perianal infection.

The most common pathogens in perianal cellulitis are aerobic gram-negative bacilli (e.g., *P. aeruginosa, K. pneumoniae, E. coli*), enterococci, and bowel anaerobes. Because of the involvement of anaerobic organisms, antibiotic coverage must include a specific antianaerobic agent such as clindamycin or metronidazole in addition to broad-spectrum aerobic coverage. Therapy should start at the first complaints of tenderness. Additional supportive measures include sitz baths three or four times daily, stool softeners, a low-bulk diet, and avoidance of unnecessary rectal manipulation, especially repetitive digital examinations. Surgical intervention should be restricted to cases that demonstrate the development of an abscess or progressive involvement of the ischiorectal fossa despite optimal antimicrobial therapy. [218,219]

## INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

### Shunt and Reservoir Infections

Children with intraventricular shunts and Ommaya reservoirs are at highest risk for central nervous system infection. The responsible pathogens are most commonly those colonizing the adjacent skin: coagulase-positive and coagulase-negative staphylococci, *Corynebacterium* sp., and *Propionibacterium acnes*; rarely, they are gram-negative bacilli. [220,221] Patients may be totally asymptomatic, in which case the diagnosis may be made by noting cerebrospinal fluid cultures repetitively positive for the same organism, or patients may have fever, headache, increased intracranial pressure, and meningismus. Most patients with Ommaya reservoir infections can be treated successfully without the need to remove the device, although this may require a combination of intrathecal and i.v. therapy. [221]

### Meningitis

Infectious meningitis in cancer patients is rare but associated with significant morbidity and mortality. [222,223] Meningitis in cancer patients can be subtle in presentation. Children with cancer presenting with fever and signs or symptoms of CNS dysfunction should be promptly evaluated. Radiographic evaluation, most commonly with a head CT scan with contrast, should be preformed if there is concern for a potential focal process. Evaluation of cerebrospinal fluid should include aerobic culture and Gram's stain, cryptococcal antigen determination, fungal culture, and cytologic analysis, in addition to the routine studies such as cell count and differential, protein, and glucose.

In a retrospective series of 40 pediatric cancer patients with meningitis, it was found that the most common risk factor was recent neurosurgical manipulation, associated with 65% of the cases.[224] Of the patients that were neutropenic, most presented with fever and altered mental status but without meningismus. The pathogens causing infection were similar to those that are commonly associated with bacteremia in this patient population, including gram-positive organisms ( *S. epidermidis*, a-hemolytic *streptococci, Enterococcus*), gram-negative organisms (*E. coli, K. pneumoniae, P. aeruginosa*), and fungi (*C. albicans* and *Aspergillus*). The primary risk factor for death related to meningitis was neutropenia at presentation.

### Encephalitis

A variety of viral, bacterial, parasitic, fungal, and rickettsial agents can be associated with encephalitis or encephalomyelitis. The relative prevalence of the various etiologic agents is altered in different populations of immunodeficient patients. For example, patients with humoral immune abnormalities, especially hypogammaglobulinemia, may have a chronic encephalitis caused by poliovirus or echovirus. [225,226] Patients with abnormalities of cell-mediated immunity more often have encephalitis caused by measles.[227] Although HSV reactivation is common during cancer chemotherapy, herpes encephalitis does not appear to be more common in immunosuppressed patients.[228] Encephalitis caused by Epstein-Barr virus, CMV, or VZV also occurs sporadically but may be more common among patients with AIDS.

Patients with encephalitis or encephalomyelitis commonly present with signs of meningeal irritation (e.g., fever, headache, nuchal rigidity) and evidence of altered mentation. Confusion may progress to stupor and finally to coma. Focal neurologic signs and seizures are relatively common. Cerebrospinal fluid examination may demonstrate a pleocytosis (10 to 2,000 cells per $mm^3$), with a predominance of mononuclear cells. An increased number of erythrocytes has been reported with HSV encephalitis. Protein levels are usually elevated, and the glucose characteristically remains within the normal range except for a decreased level in mumps infection.

For the cancer patient with focal neurologic deficits or altered mentation, it is important to separate infectious, metabolic, toxic, and neoplastic causes. CT scan or MRI of the brain may help in some instances, especially if a focal lesion is visualized, but specific diagnosis of encephalitis in an immunocompromised patient is often difficult. Acute and convalescent serum antibody titers against herpesviruses, echoviruses, and the less common arboviruses should be measured. Specific cerebrospinal fluid antibody may be detected in cases of mumps, HSV, or VZV. However, increasing experience with the technique of PCR to detect viral DNA in cerebrospinal fluid may supplant some of these older and less precise diagnostic procedures. In the past, the diagnosis of HSV encephalitis required a brain biopsy, but more recently, the application of PCR to cerebrospinal fluid has allowed for prompt and highly specific diagnosis, with excellent correlation to brain biopsy results. treatable central nervous system infection that can present as an encephalitis in an immunosuppressed child or as a mass lesion in the patient with AIDS is caused by the obligate intracellular parasite *Toxoplasma gondii*.[229] Toxoplasmosis may represent newly acquired or reactivated infection and is rarely limited to the central nervous system, usually occurring in concert with fever, lymphadenopathy, hepatitis, pneumonia, myocarditis, and pericarditis. The cerebrospinal fluid typically manifests a mononuclear pleocytosis, elevated protein, and a normal glucose concentration. A battery of serologic tests is available for the diagnosis of toxoplasmosis, but most of these are limited in their applicability to the immunosuppressed patient because of suboptimal antibody responses. Definitive diagnosis requires demonstration of the parasite within tissue sections.

Standard treatment of active toxoplasmosis is pyrimethamine combined with sulfadiazine, with the addition of folinic acid to reduce pyrimethamine-induced myelotoxicity. For immunodeficient patients, therapy should be continued for 4 to 6 weeks after the resolution of all clinical symptoms and signs. The combination of high-dose i.v. clindamycin with the usual doses of pyrimethamine appears to be a useful alternative for treatment of toxoplasmosis in patients who are unable to tolerate sulfa-based drugs. Other agents combined with pyrimethamine, such as atovaquone, dapsone, or a newer macrolide, may also be effective. [230]

### Brain Abscesses

The important differential diagnosis in a cancer patient with evidence of a focal lesion within the central nervous system is between metastatic or primary malignancy and a brain abscess. Predisposing factors for brain abscess include a contiguous infected site (e.g., otitis, sinusitis, dental abscess), a history of penetrating cranial trauma, congenital cardiac disease, bacterial endocarditis, and pulmonary infection. In addition to the usual aerobic and anaerobic bacteria responsible for abscesses in immunocompetent patients, fungal and nocardial species are particularly prone to cause disease in immunosuppressed patients. These infections are usually associated with pulmonary infiltrates.

Early evaluation and specific diagnosis are crucial in the management of brain abscess, because effective antimicrobial or neurosurgical therapy is available. Diagnosis is commonly made by radiographic demonstration of a localized mass and followed by an open or closed procedure to aspirate or resect the localized lesion.

## INFECTIONS OF THE GENITOURINARY TRACT

The genitourinary tract is infrequently the source of infection in the immunocompromised child. However, local obstruction resulting from tumor, neurologic dysfunction mediated by spinal cord compression or medications (e.g., vincristine, narcotics), and local therapeutic maneuvers (e.g., radiotherapy, surgery, bladder catheterization) can predispose cancer patients to genitourinary infection. Most commonly, gram-negative aerobic bacilli (e.g., *E. coli, Klebsiella* sp.,*Proteus* sp., *P. aeruginosa*) or enterococci are the causative agents.

In a neutropenic patient, urine culture of a single organism should prompt antibiotic intervention whether or not the patient is symptomatic. The presence or absence of leukocytes in the urine must not be relied on as a diagnostic criterion in the neutropenic patient.

Differentiation between colonization and tissue invasion is particularly difficult for fungal pathogens. Fungal colonization is especially prevalent among patients with indwelling urinary catheters and those receiving broad-spectrum antimicrobial therapy. Unlike the typical situation with bacterial pathogens, in which clinical signs and symptoms are apparent, fungal invasion of the genitourinary tract may be insidious. The repetitive isolation of a particular fungal species (usually *C. albicans, Candida tropicalis*, or *Candida glabrata*) in association with fever, deteriorating renal function, or, rarely, flank pain should prompt the institution of systemic AMB. Heavily

colonized bladders or superficial bladder infections manifested by persistence of positive urine cultures despite removal of predisposing factors may be effectively treated by fluconazole, which is highly concentrated in the urine.

Adenovirus, particularly type 11, and polyomavirus (BK virus) have been associated with hemorrhagic cystitis in bone marrow transplant recipients. Bladder pain and gross hematuria may occur suddenly and can be very difficult to control. The occurrence of aggressive adenovirus nephritis causing renal failure and ultimate mortality has been described as well. Urinary tract infections may be localized, but in some cases they precede a disseminated adenovirus infection. Detection of virus as well as defining serotype is possible by PCR. [231] Case reports of successful control of adenoviral and polyomavirus hemorrhagic cystitis with i.v. ribavirin, vidarabine, and ganciclovir have yet to be confirmed by larger studies. [232,233,234 and 235]

## INFECTIONS OF THE CARDIOVASCULAR SYSTEM

Cardiovascular infections are relatively uncommon among cancer patients, probably because of the early institution of broad-spectrum antimicrobial therapy. However, cancer patients who have predisposing factors for a cardiovascular infection, such as dental abscess, a history of i.v. drug abuse, or congenital cardiac anomaly, are at risk. Endovascular infections are more likely with the increased use of indwelling venous access catheters. Although gram-positive bacterial species (e.g., *Enterococcus*, viridans or b-hemolytic streptococci, *S. aureus*) most commonly cause endovascular infections, aerobic gram-negative bacilli and fungi may also cause disease. These latter pathogens are particularly difficult to eradicate, and morbidity and mortality remain discouragingly high.

The clinical manifestations of endocarditis in the immunosuppressed patient are similar to those in an immunocompetent patient. Nonspecific complaints of fever, chills, malaise, fatigue, night sweats, and weight loss are common, but these complaints are nondescript, and the degree of diagnostic specificity that may be ascribed to them is slight. In most instances, the diagnosis must be made on the basis of the physical and laboratory evaluations. The numerous physical stigmata of endocarditis (e.g., heart murmurs, splinter hemorrhages, Roth's spots, splenomegaly) should be sought, but the diagnosis is confirmed by the isolation of an organism from multiple blood cultures.

The complications of endovascular infection in immunocompromised patients are similar to those described for patients without cancer. Valvular insufficiency resulting in congestive heart failure, emboli, and renal failure is the most serious. Fungal endocarditis is particularly likely to cause large-vessel embolization. [236] Patients with *Candida* or *Aspergillus* endocarditis are treated with valve replacement and AMB.

Therapy must be directed at the specific pathogen. The isolation of *S. aureus* or *S. epidermidis* from multiple blood samples, even if the patient has an indwelling catheter, is not a sufficient criterion for prolonged antibiotic therapy unless a valvular infection can be confirmed. A standard 10- to 14-day course of therapy suffices for these patients.[141]

## INFECTIONS OF THE SKIN

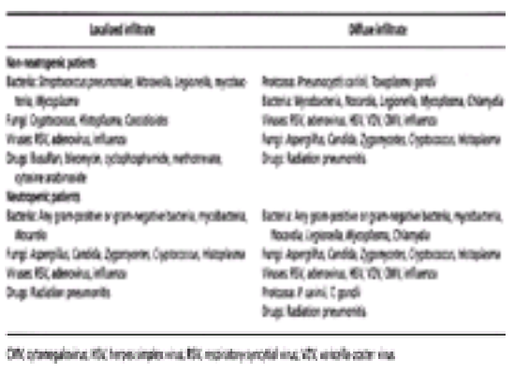
The skin can be infected primarily or in association with bacteremia (e.g., *P. aeruginosa*, *Aeromonas hydrophila*, *S. marcescens*), fungemia (*Aspergillus*, *Candida*, *Trichosporon*, *Fusarium*, and *Cryptococcus*), or viremia (e.g., HSV, VZV, and CMV). Skin lesions may permit the early diagnosis of an established infection, and new lesions should be aspirated or biopsied and the material stained (e.g., Gram's stain, wet mount, and methylene blue) and cultured. If the lesions are vesicular, the base should be scraped, smeared on a glass slide, and submitted for a direct fluorescent antibody test to detect HSV or VZV. Alternatively, a Tzanck preparation may be performed to look for the multinucleated giant cells that are characteristic of these viral infections.

Primary varicella is a significant concern for the child with cancer, because the mortality rate in untreated patients ranges from 7% to 20%, usually owing to visceral dissemination to the liver, lung, and central nervous system. [237] Severe abdominal pain, back pain, or evidence of inappropriate antidiuretic hormone secretion may herald multisystem involvement, indicating the need for prompt use of acyclovir. [238] High doses of i.v. acyclovir (500 mg per $m^2$ every 8 hours) are indicated for the treatment of primary varicella or herpes zoster in very immunosuppressed patients. [239]

Scabies infestation may present as papules, excoriations, or vesicles located particularly in the interdigital spaces and on the palms, soles, face, neck, and scalp. A severe variant of scabies, Norwegian or crusted scabies, occurs in immunodeficient patients and is characterized by widespread, hyperkeratotic, crusted lesions. These lesions contain heavy burdens of mites and their eggs, and Norwegian scabies is highly contagious. Lindane 1% lotion has been the standard treatment for scabies, although it is not recommended for young children because it is cutaneously absorbed and may cause neurologic side effects. Permethrin 5% cream is poorly absorbed and may be more effective than lindane, so it is recommended for children. The antiparasitic agent, ivermectin, has been shown to be highly effective in curing both routine cases and Norwegian scabies after a single oral dose; the simplicity and efficacy of this therapy may preclude use of the topical agents. [240]

## PREVENTION OF INFECTION IN CHILDREN WITH CANCER

In a multitude of clinical trials investigating the efficacy of various measures to prevent or reduce infection, the most important antiinfective measure identified has been the simplest: careful hand washing practices.[241,242] Several approaches have been taken to decrease the acquisition of new organisms or to suppress those already colonizing the cancer patient (Table 41-5). However, no method has stood out as singularly effective and each has both promise and problems.



CMV, cytomegalovirus; HSV, herpes simplex virus; RSV, respiratory syncytial virus; VZV, varicella-zoster virus

**TABLE 41-5. CAUSES OF PNEUMONIA IN CANCER PATIENTS**

### Preventing the Acquisition of New Organisms

Because most of the organisms responsible for infections in patients with cancer are derived from the endogenous flora, and almost half of this flora is acquired from the hospital environment, much attention has been directed toward preventing the acquisition of potential pathogens. Inanimate objects within the hospital environment (e.g., faucet aerators, showerheads, respirators, plants, and floors) are reservoirs of pathogenic organisms. Although epidemiologic studies have for the most part investigated nonimmunocompromised patients, they do suggest that transmission from inanimate sources usually requires a human vector. Therefore, the most efficacious intervention that can be performed is adherence to strict hand washing precautions. The easiest way to enforce such a policy is to educate the child and parents to disallow contact with anyone who has neglected to wash his or her hands.

A second maneuver to decrease the acquisition of new organisms is to maintain a cooked diet during periods of granulocytopenia, with avoidance of fresh fruits and vegetables and unprocessed dairy products, because these foods are naturally contaminated with gram-negative bacteria, especially *K. pneumoniae*, *E. coli*, and *P. aeruginosa*.[243,244]

Environmental sources can contribute to fungal (especially *Aspergillus*) and bacterial (*Legionella*) colonization and infection. In medical centers in which *Aspergillus* is

a significant problem, special air filtration systems such as high-efficiency particulate air filters (HEPA filters) or water purification systems may be helpful.

Although the technique of reverse isolation has often been used, it does not significantly reduce the acquisition of new organisms in an environment in which hand washing techniques are strictly followed. There is no compelling reason to enforce this policy, particularly because the extra expense, time consumption, and inconvenience are not balanced by a beneficial effect. [245,246]

Total protective isolation is a comprehensive regimen designed to reduce the patient's endogenous microbial burden while preventing the acquisition of new organisms (Table 41-5). A sterile environment is created in a clean-air room with constant positive-pressure airflow. It is maintained by an aggressive program of surface decontamination and sterilization of all objects that enter the room and by an intensive regimen to disinfect the patient, including oral nonabsorbable antibiotics, skin antiseptics, antibiotic sprays and ointments, and a low-microbial diet. The total protective environment does reduce the number of infections in profoundly granulocytopenic patients. It is expensive, however, and because of the improvement in treating established infections, it does not offer a survival advantage to patients. Total protective isolation is not necessary for the routine care of cancer patients. Modifications of the approach are used, on occasion, for patients undergoing allogeneic bone marrow transplantation and for patients who are likely to experience periods of 30 or more days of profound neutropenia. [246]

## PROPHYLACTIC ANTIBIOTICS

### Antibacterial Prophylaxis

Because the GI tract is the source of many of the pathogens causing microbiologically defined infections, investigators initially evaluated the efficacy of reducing the endogenous GI flora with oral nonabsorbable antibiotics (e.g., vancomycin, gentamicin, polymyxin B, nystatin, framycetin, colistin). This technique has not been especially valuable because the agents used are unpalatable and poorly tolerated, making compliance a significant problem, especially among patients receiving emetogenic chemotherapy. Equally disturbing has been the emergence of resistant bacterial strains among patients receiving aminoglycoside-containing regimens. Therefore, prophylactic regimens aimed solely at reducing the total endogenous GI flora cannot be recommended. [247]

A modified technique is selective decontamination of the GI tract with antibiotics that preserve the anaerobic flora while reducing the aerobic bacteria. This approach is based on experimental data showing that preservation of the anaerobic flora of the GI tract provides colonization resistance against aerobic and fungal organisms.[248,249] The most commonly investigated agent for selective decontamination has been TMP/SMX. Early trials of this drug in children and adults demonstrated a reduction in all infections and in bacteremic episodes. Many follow-up clinical trials have yielded conflicting results, however, perhaps because of variability in study design, nonuniform patient populations, and failure to monitor compliance properly. The potential for reduction in infectious morbidity and mortality must be balanced against two important adverse effects observed with the prophylactic use of TMP/SMX: the prolongation of granulocytopenia and the emergence of resistant organisms. Successful selective decontamination of the GI tract requires excellent patient compliance and close microbiologic monitoring to adjust the antimicrobial regimen properly for resistant or newly emerging species. Strict compliance with the oral regimens is often difficult, however, and surveillance cultures are costly in time and money.[250,251,252,253 and 254]

The fluoroquinolone antibiotics are attractive for oral prophylaxis because of their bioavailability, excellent tolerability, and broad spectrum. These agents have been widely used in adult oncology patients but are not generally used for prophylaxis in children because of concern of cartilage toxicity with long-term exposure. In adults, comparative studies have shown no advantage of the oral quinolones over more traditional regimens of infection prophylaxis such as for prevention of infection. [255,256] Moreover, none of the studies of antibacterial prophylaxis has demonstrated a reduction in mortality caused by infection. Further, prophylaxis with fluoroquinolones is not recommended by the IDSA.

### Antifungal Prophylaxis

Because of the increasing incidence of invasive mycoses in immunocompromised hosts, antifungal prophylaxis also has been extensively studied. The topic of antifungal prophylaxis in neutropenic patients has been reviewed in detail elsewhere. [257] Orally administered andtopically applied nystatin, AMB, miconazole, and clotrimazole, as well as systemically absorbed ketoconazole, fluconazole, and itraconazole have all been evaluated as prophylactic antifungal agents in neutropenic patients. Most prophylactic regimens have been aimed at reducing invasive infections caused by *Candida* sp. and, by virtue of the antifungal activity of the agents used, would not be expected to have a significant impact against *Aspergillus* or filamentous fungal pathogens. Data from laboratory studies of fluconazole were particularly compelling for the use of fluconazole for prevention of invasive candidiasis in neutropenic hosts. [258]

Several randomized, placebo-controlled studies demonstrated that the prophylactic administration of fluconazole to recipients of allogeneic bone marrow transplant recipients reduced the incidence of both disseminated and mucosal candidiasis. [258,260] These and other studies using prophylactic antifungal regimens observed a shift in the colonization pattern of fungal organisms, usually toward more resistant fungi. [261] The prophylactic regimens may eradicate the susceptible fungi while permitting overgrowth and ultimate invasion by more resistant species, including *C. glabrata*, *Candida krusei*, *Candida parapsilosis*, *Aspergillus*, and other filamentous species of fungi. Fluconazole is most beneficial in prevention of disseminated candidiasis in the neutropenic allogeneic bone marrow transplant recipient. The decision to use fluconazole or other prophylactic antifungal agents in other patient populations depends on the institution, the cytotoxic regimen used, and the patient. [262]

### Antiviral Prophylaxis

Several antiviral agents can be used for selective prophylaxis. Amantadine has proved prophylactic activity against influenza A (although not against influenza B) in school children treated with 100 mg per day, and it is likely to be similarly efficacious in the immunocompromised host. [263] It may be particularly useful if given to a susceptible immunocompromised individual for a prolonged period during an influenza A outbreak or during “flu season.” Rimantadine has equivalent protective efficacy but is associated with fewer central nervous system effects than is amantadine. Postexposure prophylaxis of family members is also effective with both drugs, although it is recommended that simultaneous prophylaxis and treatment of influenza A be avoided within a household so that resistant virus strains do not become a significant problem.[264] The use of the newer neuraminidase inhibitors in immunocompromised patients as prophylactic agents has not yet been studied.

Acyclovir, given orally or i.v., is effective prophylaxis against reactivations of HSV in seropositive bone marrow transplantation patients and in those undergoing intensive chemotherapy for acute leukemia.[265,266] Doses ranging from 250 mg per $m^2$ i.v. every 8 hours to 5 mg per kg i.v. every 12 hours, and oral doses of 400 mg given three times daily are effective in preventing reactivation of HSV in seropositive individuals. Although acyclovir is therapeutically less active against VZV or CMV, prophylactic acyclovir given to bone marrow transplant recipients may nonetheless decrease the occurrence of zoster and invasive CMV disease during the posttransplant period.[267,268]

Acyclovir-resistant strains of HSV have been recognized with increasing frequency, particularly in patients with HIV infection. In bone marrow transplantation patients and in those with HIV infection, resistant HSV is associated with indolent disease, characterized by the persistence of low-grade, chronic mucocutaneous lesions that may be painful and refractory to routine or even high-dose acyclovir therapy. The clinical presentation of resistant HSV infection is preceded by a prolonged continuous or intermittent course of acyclovir, such as a prophylactic regimen. Discontinuation of acyclovir often allows the reemergence of a sensitive virus strain from the mixed pool of latent virus. Refractory lesions may be responsive to foscarnet. [269]

Ganciclovir prophylaxis can reduce the frequency of invasive CMV disease in patients who have received bone marrow transplants. The marked myelosuppressive effects of ganciclovir are problematic for most patients, however, making it an unattractive routine prophylactic agent. Targeting of those who are at highest risk for severe CMV disease has yielded the practice of “preemptive” ganciclovir therapy—that is, treatment of patients who have evidence of CMV reactivation in surveillance assays (CMV antigenemia, CMV PCR). This approach has been shown to effectively suppress CMV culture positivity in most patients and, accordingly, it is associated with dramatically fewer cases of invasive CMV disease. [270,271]

### *Pneumocystis* Prophylaxis

Successful prophylaxis for PCP was originally described in high-risk oncology patients. [121] In the 1980s PCP emerged as the most common opportunistic infection in individuals with AIDS. Most large studies of prophylactic regimens since that time have been undertaken in the HIV population, with results then abstracted for use in other immunocompromised groups.

The decision to administer antimicrobial prophylaxis to oncology patients should be based on underlying disease and the type and intensity of immunosuppressive therapy. The reported incidence of PCP in patients before the routine use of prophylaxis was 22% to 43% in children with ALL, 25% in patients being treated for rhabdomyosarcoma,[121] 16% of those having undergone a bone marrow transplant[272] and 27% of those children with severe combined immunodeficiency syndrome. In some centers almost half of the cases of PCP occur in patients with solid tumors.[273] In addition, patients with brain tumors receiving significant doses of corticosteroids are also at risk.[274]

There are several effective regimens and the choice between them often depends on the patient's tolerance of their various side effects. TMP-SMX, b.i.d for 3 days per week should in general be considered the first-line regimen.[275] For patients who can tolerate this regimen, the failure rate is near zero.[276] The use of TMP-SMX is limited, however, in a significant number of individuals by rash, neutropenia, and GI symptoms. The overall rate of treatment-limiting adverse reactions is variably reported but is estimated at approximately 19 per 100 patient years.[276]

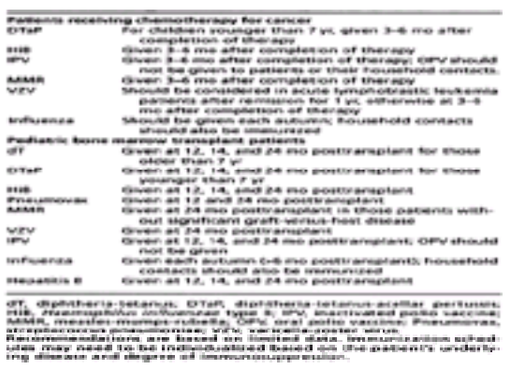
Dapsone administered at a dose of 2 mg per kg per day (with a maximum dose of 100 mg per day) is also effective therapy. The rate of reported failure of prophylactic dapsone ranges from 0% to 21%.[123] The side effects of dapsone include rash, anemia, methemoglobinemia, agranulocytosis, and hepatitis. The incidence of treatment-limiting adverse reactions is estimated to be similar to that of TMP-SMX.

Aerosolized pentamidine administered monthly is also effective therapy, having a comparable failure rate to dapsone.[276] Its primary side effect is bronchospasm at the time of administration. The use of aerosolized pentamidine is often limited in very young children because of technical and compliance issues. Intravenous pentamidine has not undergone sufficient study as a prophylactic regimen to allow it to be recommended.

Atovaquone has been shown to be as effective as dapsone in a single large trial of adult patients with HIV who were intolerant of TMP-SMX.[277] In addition, in patients not already receiving dapsone at the time of randomization it was better tolerated. The most common adverse events include upper GI symptoms and diarrhea. Pharmacokinetic studies have been performed in children with HIV giving rise to dosing recommendations of 30 mg per kg per day in children aged 1 to 3 months and older than 24 months and 45 mg per kg per day for those aged between 3 and 24 months. These doses were chosen to attain a serum level of 15 µg per ml, which in adult patients is associated with therapeutic success of greater than 95%.[129]

## IMMUNIZATION

Immunizations are an important part of the care of healthy children. The routine vaccination schedule is often disrupted for younger children undergoing therapy for cancer. No universally accepted recommendations for immunizing children undergoing therapy for cancer exist, but some general guidelines can be applied ( Table 41-6). The American Academy of Pediatrics and the Centers for Disease Control and Prevention regularly publish updated guidelines regarding immunization practices in healthy and immunocompromised patients.[278,279] In considering reasonable vaccine strategies, information about the host's risks for infection need to be balanced with the safety and efficacy of each vaccine in this population. Two main concerns have to be considered: (a) will the patient be able to mount (or maintain) an antibody response, and (b) could the vaccine itself (in the case of attenuated live organisms) cause disease?[280]



| | |
|---|---|
| **Patients receiving chemotherapy for cancer** | |
| DTaP | For children younger than 7 yr, given 3–6 mo after completion of therapy |
| HIB | Given 3–6 mo after completion of therapy |
| IPV | Given 3–6 mo after completion of therapy; OPV should not be given to patients or their household contacts. |
| MMR | Given 3–6 mo after completion of therapy |
| VZV | Should be considered in acute lymphoblastic leukemia patients after remission for 1 yr, otherwise at 3–6 mo after completion of therapy |
| Influenza | Should be given each autumn; household contacts should also be immunized |
| **Pediatric bone marrow transplant patients** | |
| dT | Given at 12, 14, and 24 mo postransplant for those older than 7 yr |
| DTaP | Given at 12, 14, and 24 mo postransplant for those younger than 7 yr |
| HIB | Given at 12, 14, and 24 mo postransplant |
| Pneumovax | Given at 12 and 24 mo postransplant |
| MMR | Given at 24 mo posttransplant in those patients without significant graft-versus-host disease |
| VZV | Given at 24 mo posttransplant |
| IPV | Given at 12, 14, and 24 mo posttransplant; OPV should not be given |
| Influenza | Given each autumn (>6 mo posttransplant); household contacts should also be immunized |
| Hepatitis B | Given at 12, 14, and 24 mo posttransplant |

dT, diphtheria-tetanus; DTaP, diphtheria-tetanus-acellular pertussis; HIB, Haemophilus influenzae type b; IPV, inactivated polio vaccine; MMR, measles-mumps-rubella; OPV, oral polio vaccine; Pneumovax, streptococcus pneumoniae; VZV, varicella-zoster virus.
Recommendations are based on limited data. Immunization schedules may need to be individualized based on the patient's underlying disease and degree of immunosuppression.

**TABLE 41-6. IMMUNIZATIONS IN PEDIATRIC CANCER PATIENTS**

### Live Virus Vaccines

Measles-mumps-rubella is a live virus vaccine that is contraindicated in patients undergoing active chemotherapy. In the early development of the vaccine one of eight children vaccinated with the Edmonton b strain measles vaccine died of the disease. Immunization has been shown to be safe and is recommended for patients with ALL after completion of therapy, with the usual waiting period of 3 to 6 months to allow for T-cell reconstitution. It has been recommended that patients having completed therapy for Hodgkin's disease not be vaccinated given their prolonged T-cell deficits.[281] Household contacts can be safely vaccinated because transmission of the vaccine virus does not occur.[278] The risk associated with wild-type measles is considered to be higher than the risk for vaccine-related complications.[282,283]

Oral polio vaccine is a live virus vaccine that is contraindicated in immunocompromised patients and their household contacts.[278] In activated polio vaccine can be used safely during treatment for nonimmunized patients. Reimmunization after the completion of therapy with inactivated polio vaccine is generally recommended.[281]

The varicella vaccine is potentially the most extensively studied vaccine in immunocompromised children. Varicella vaccine is now universally recommended for healthy individuals in early childhood and for susceptible older children and adolescents. It is not contraindicated in household contacts of immunosuppressed individuals.[278] The use of varicella vaccine has been studied extensively in children with ALL, and it has been shown to be safe with the most common toxicity being a mild rash occurring in 50% of those treated.[284] The efficacy of the vaccine has been shown in the degree of protection from acquiring the disease from household contacts, with 14% developing mild disease and complete protection from severe varicella. There has been concern about herpes zoster in immunocompromised vaccinees, but it has been shown that leukemic vaccinees are less likely to develop zoster than are comparable children with leukemia who had wild-type infection.[284,285] Passive immunoprophylaxis either with varicella zoster immune globulin within 96 hours of exposure or regular infusions of gamma globulin may be indicated in the child at high risk.

### Live Bacteria Vaccines

The only common live bacterium that potentially could be used in a childhood immunization schedule is the bacillus Calmette-Guérin (BCG) vaccine. Although not used in the United States, it is still recommended in more than 100 countries worldwide.[278] Because disseminated bacillus Calmette-Guérinitis can occur in immunocompromised children, it is generally contraindicated in that population.[286,287,288] and [289]

### Inactivated Bacteria, Inactivated Viruses, Polysaccharide-Protein Conjugates, and Toxoids

Diphtheria-pertussis-tetanus vaccine has been evaluated in young infants with neuroblastoma and in children receiving maintenance therapy for various malignancies, and these children have been shown to mount adequate responses.[290,291] It has been suggested that children should be given this vaccine at scheduled times even while undergoing active therapy. The alternative approach is to immunize at the end of therapy. To decrease the risk of seizures (a special concern in children with brain tumors or preexisting seizure disorders) it is currently recommended that all children should receive the acellular form of the pertussis component (DtaP).

Patients with ALL as well as those with Hodgkin's disease, treated at a time when splenectomy was routine, have been shown to be at a higher risk for invasive

pneumococcal and *H. influenzae* type B disease.[292,293 and 294] Pneumococcal infection contributes significantly to the morbidity and mortality of pediatric HIV infection.[295,296] The polysaccharide pneumococcal vaccine is only moderately immunogenic when studied in oncology or transplantation populations. Evaluation of the newer pneumococcal vaccines needs to be carried out in children with cancer; however, it is currently recommended that patients who are expected to be functionally or anatomically asplenic should receive pneumococcal vaccine.

The *H. influenzae* type B conjugate vaccines have been tested in children who were on treatment or who had completed treatment for ALL, as well as in children with HIV infection. The responses were not normal, but those that did respond had protective antibodies that were measurable for 12 months. [292,297,298] Although the data are not definitive, some authors recommend the vaccination of *all* immunosuppressed individuals with pneumococcal, *H. influenzae* type B, and meningococcal vaccines.[279,281]

The efficacy of the influenza vaccine in patients undergoing cancer therapy is controversial. In the pediatric age group it was shown in a small retrospective study of patients with ALL that immunization decreased the incidence of influenza infection compared to nonimmunized controls. [299] Despite the lack of definitive data, it is generally recommended that all immunocompromised children and their household contacts receive yearly influenza vaccines before flu season. [279]

## GRANULOCYTE TRANSFUSIONS

In the 1970s there was significant interest in the use of granulocyte transfusions for the treatment of patients with severe infections and neutropenia. Support for this modality waned as results from studies revealed mixed efficacy and occasional severe toxicity.

Between 1972 and 1982 there were a number of randomized controlled studies published evaluating the efficacy of granulocyte transfusions. Some studies showed a clinical benefit for the neutrophil transfusion group compared to controls, [300,301 and 302] some showed no overall benefit but efficacy in certain subgroups of patients, [303] and some reported no benefit.[304] On further analysis of these studies, one potential explanation for the varying results was the use of varying cell dose, with studies using higher doses showing efficacy and those using a smaller cell dose (i.e., less than 0.4 to 0.5 × $10^{10}$ PMNs transfused) being less effective.[305]

Recent technical advances, with the possibility of collecting significantly larger numbers of PMNs per pheresis, have made a reevaluation of this modality of interest.[306,307] It is estimated that an adequate cell dose for a single granulocyte transfusion should be greater than 1 × $10^{10}$ PMN cells.[308] Previously, the collection of adequate numbers of cells from normal donors proved to be difficult, even with corticosteroid stimulation. More recently, the use of granulocyte colony-stimulating factor (G-CSF) to mobilize granulocytes in normal healthy donors has been shown to be safe and effective, allowing for the collection of significantly more PMN cells per cycle of pheresis.[309,310]

Cell collection techniques have also dramatically improved. Cell collection is now done by centrifugation as opposed to filtration. The process of filtration has been shown to activate neutrophils and lead to smaller cell yields and higher rates of toxic reactions, presumably related to activated white blood cells, whereas centrifugation yields higher numbers of cells with potentially less infusion-related toxicity.

The use of “matched” donors has been suggested as an important factor in granulocyte transfusion efficacy. [306] There is evidence that alloimmunization to HLA antigens decreases cell recovery in the recipient and may also correlate with the incidence of transfusion reactions. [311,312] There is clearly a need for further randomized controlled trials to reevaluate efficacy and safety of this modality given the progress made in pheresis and transfusion medicine. Studies using adequate cell dose and matching techniques are imperative.

## CYTOKINES

The development of cytokines for adjunctive therapy in oncology patients was greeted with great enthusiasm. The potential ability of the colony-stimulating factors (CSFs) to attenuate the marrow toxic effects of cancer chemotherapy, radiotherapy, and bone marrow transplantation was hoped to have a large impact on infectious morbidity and mortality. CSFs have come to have an important role in the care of the oncology patient, although it is clear that they should be used judiciously to maximize medical benefit, limit potential toxicity, and be cost-effective.

The two cytokines that have undergone the most intense study for their potential to decrease the infectious morbidity of chemotherapy are G-CSF (filgrastim) and granulocyte-macrophage-CSF (GM-CSF; sargramostin). G-CSF promotes the proliferation and maturation of neutrophilic precursors and the function of mature neutrophils. GM-CSF additionally enhances the number and function of cells of the monocyte-macrophage lineage. There are limited data comparing the two agents directly in terms of their relative efficacy and toxicity, [313] which in general are considered similar. The recommended use of a given agent varies, primarily based on the indication for which it was developed. [314]

The doses of the two agents are 5 µg per kg per day for G-CSF and 250 µg per $m^2$ per day for GM-CSF. The use of higher doses has not been associated with improved clinical outcome.[315,316] The exception to this is in the setting of donor stimulation for peripheral blood stem cell pheresis, in which there may be an advantage to the use of 10 µg per g per day of G-CSF.[317,318,319 and 320] In either setting, rounding the dose to the closest vial size is likely to save costs without detriment to the patient. The CSFs should be administered subcutaneously but may also be given i.v.

In 1994 the American Society for Clinical Oncology (ASCO) published the first set of recommendations for the appropriate use of growth factors based on an extensive review and analysis of the relevant literature. [314] These recommendations were updated in 1997.[321]

### Primary Prophylaxis: Use of Colony-Stimulating Factors in the Prevention of Fever and Neutropenia

The use of CSFs for primary prevention involves their administration immediately following a course of myelotoxic chemotherapy in an attempt to decrease the depth and duration of neutropenia and the associated risk of infection.

There is evidence from a number of studies that the use of CSFs in patients receiving significantly myelotoxic chemotherapy can reduce the incidence of fever and neutropenia. In addition, for patients receiving prophylactic CSF, the duration of neutropenia, antibiotic administration, and hospitalization may be shortened.[322,323,324,325,326 and 327] Results regarding the effect of incidence of documented infection have been more variable. Importantly, no study to date has shown a significant effect of disease-free or overall survival.

The use of G-CSF as primary prophylaxis for patients undergoing less aggressive chemotherapeutic regimens with a low incidence of fever and neutropenia is of unclear medical or economic benefit. If the number of patients receiving the cytokine injections far exceeds those who would potentially experience any beneficial effects, the expense of the growth factor and the discomfort of daily injections in all patients could offset the advantage to a few patients.

The ASCO guidelines recommend the use of CSF for primary prophylaxis if the anticipated rate of fever and neutropenia for a given chemotherapeutic regimen is greater than 40%.[321] This degree of myelosuppression and associated fever is relatively rare in adult oncology, but may be more common in pediatrics, in which dose-intensive therapy is common. In addition to those groups of patients with anticipated high rate of fever and neutropenia, there may be individuals who may benefit from primary prophylaxis, such as patients who have received extensive prior chemotherapy or radiation therapy to their pelvis or spine and are therefore expected to have more significant myelotoxicity from any given regimen.

End points for G-CSF discontinuation have varied according to different protocols, but the goal should be to maintain the peripheral leukocyte count at protective levels until the intrinsic bone marrow activity is sufficient to independently achieve these levels. Although investigators have targeted total leukocyte counts of 10,000 cells per mL as a stopping point for cytokine administration, lower end points are being explored to improve the cost-effectiveness of this intervention.

A survey of clinicians in the Pediatric Oncology Group indicated that CSFs are used for primary prophylaxis more commonly in children than in adults. [328] This was in part due to the fact that, unlike their adult counterparts, the majority of pediatric patients are on clinical research protocols that often require or suggest growth factors use.

### Secondary Prophylaxis: Use of Colony-Stimulating Factors in Patients with a Prior Episode of Fever and Neutropenia

The restriction of CSF administration to patients with a history of a prior episode of fever and neutropenia may provide a more select patient population that may benefit from CSFs. In addition, this strategy may avoid their use, and associated toxicities and costs, in a group of patients for which they would provide limited or no benefit. The ASCO recommendations encourage chemotherapy dose adjustment for subsequent cycles of therapy for patients that have developed fever and neutropenia on a prior cycle unless there is evidence that maintaining dose intensity is beneficial to patient outcome. If maintaining dose intensity is important, CSFs should be considered for secondary prophylaxis. Interestingly, in the Pediatric Oncology Group survey of practicing pediatric oncologists, dose modification was rarely selected as a mode of secondary prophylaxis and conversely, CSF use was common.[328]

### Tertiary Prophylaxis: Use of Colony-Stimulating Factors in Patients with Known Neutropenia but without Fever

There have been two small studies and one large trial of the initiation of CSFs at the time of a patient's diagnosis with neutropenia. [329],[330] and [331] Although the number of days of neutropenia was shortened, there was no measurable clinical benefit in terms of days in the hospital, days of antibiotic therapy, or documented infections. Given these data it is recommended that CSFs not be routinely initiated for patients presenting with neutropenia alone.

### Use of Colony-Stimulating Factors for the Adjunctive Treatment of Fever and Neutropenia

There have been a series of prospective, randomized, controlled trials [332],[333],[334],[335],[336],[337],[338] and [339] addressing the question of whether administration of G-CSF or GM-CSF as adjunctive therapy to antibiotics, beginning at the time of diagnosis of fever and neutropenia, has beneficial effects on patient outcome. In general, as in the use in other circumstances, CSFs have been shown to decrease the duration of neutropenia and have variable effects on days of fever, days of antibiotic administration, and days of hospitalization. There was no measurable impact on infection-related mortality. In terms of cost savings, the use of CSFs in this situation has also shown mixed results, with some studies showing reduced costs,[333],[339] whereas others showed increased costs in the CSF-treated groups.[334],[335] and [336] The ASCO recommendations are that CSFs not routinely be initiated as adjunctive therapy for the patient presenting with fever and neutropenia. [321]

There may be a group of high-risk febrile neutropenic patients for which the initiation of CSFs may be of benefit. This may include a group with profound or prolonged neutropenia, patients with prior severe infections, or in elderly or debilitated patient groups. There is not sufficient data to date to assess whether the use of CSFs at the time of fever and neutropenia in these situations is clinically beneficial.

### Use of Colony-Stimulating Factors in Documented Infections in Immunocompromised Hosts

G-CSF and GM-CSF can enhance antimicrobial activity of granulocytes and monocytes *in vitro*. Accordingly, they may augment microbicidal activity of these effector cells in the immunocompromised patient and may therefore be useful as adjuncts to antimicrobial agents in the treatment of ongoing infections. Although there are some data from animal models to suggest a survival benefit of CSFs given during septic episodes, one small clinical study in leukemic children showed no benefit to the delayed addition of G-CSF at the time of documented sepsis.[340],[341] Based on current therapy recommendations, it is most likely that patients who are at highest risk for sepsis will already be receiving CSF prophylaxis during neutropenia.

### Use of Colony-Stimulating Factors in Stem Cell Transplantation

Both G-CSF and GM-CSF have been shown to significantly accelerate neutrophil recovery after autologous and allogeneic bone marrow and peripheral blood stem cell transplantation.[342],[343],[344],[345],[346],[347] and [348] The effects on the incidence of fever or documented infections, antibiotic usage, or duration of hospitalization have been more variable. Furthermore, these cytokines do not augment platelet recovery, which remains a significant problem for transplant recipients. Nonetheless, CSFs are used as part of most immediate posttransplant regimens for their impact on shortening the duration of neutropenia. Whether they affect long-term outcomes, such as graft failure or survival, is still not apparent. Generally, cytokine therapy is continued until engraftment is documented and an adequate circulating neutrophil count has been observed on successive days. The doses, routes, and schedule of administration are similar to those used in chemotherapy-induced neutropenia. In selected circumstances, it may be reasonable to consider the use of a hematopoietic growth factor in patients who are slow in neutrophil engraftment or who demonstrate an inadequate recovery after autologous or allogeneic transplantation. [321]

## FUTURE DIRECTIONS

With the evolution of new cancer therapies there will continue to be new challenges in the assessment and management of infections in children with cancer. The use of novel chemotherapeutic agents, such as monoclonal antibodies and anti-angiogenic agents will provide new challenges in the assessment of their impact on the child's immune function and the associated infectious complications.

In addition to novel chemotherapeutic agents, there are numerous new antimicrobial agents in the process of development. These drugs will need to be evaluated not only for safety and efficacy but also for cost-effectiveness and ease of administration. It is critical that as new drugs are developed, knowledge about their appropriate use in children is obtained.

Further understanding of the components and function of the immune system in the “normal” host and how it is affected by cancer and its therapy will continue to inform the best clinical care. Understanding risk factors for infection, the spectrum of clinical presentations of infectious disease in different hosts, and the appropriate use of diagnostic tests and therapeutic agents will continue to be challenges in the care of the child with cancer.

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# NUTRITIONAL SUPPORTIVE CARE

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## INTRODUCTION

The high prevalence of undernutrition in adult [1,2] and pediatric[3] cancer patients has been appreciated for decades and continues to be documented. [4,5] Although the prognostic significance of malnutrition among patients with cancer is controversial, [6,7] it is generally accepted that nutritional support is an important component of medical therapy (Table 42-1). Indeed, because of the unique nutritional needs of pediatric patients, [8] including energy needs for growth and development, randomized, controlled studies have been difficult to perform concerning this inherently vital supportive therapy. Parents of children with cancer are quite concerned about issues of appetite and other gastrointestinal symptoms, even when death is imminent.[9] The frequent use of complementary and alternative dietary supplements among families with cancer also underlines the importance that patients attach to nutritional therapy. [10] This chapter reviews the definitions, epidemiology, etiology, and practical therapy of nutritional problems of the pediatric cancer patient.

**TABLE 42-1. RATIONALE FOR NUTRITION SCREENING AND INTERVENTION IN PEDIATRIC CANCER PATIENTS**

## DEFINITION OF CANCER CACHEXIA

Cachexia has been classically defined as a severe state of malnutrition characterized by anorexia, weight loss, muscle wasting, and anemia. [11,12] In its broader sense, cancer cachexia has also been used to describe a variety of metabolic phenomena, including hypoalbuminemia, hypoglycemia, lactic acidosis, hyperlipidemia, impaired hepatic function, glucose intolerance with insulin resistance, elevated gluconeogenesis, skeletal muscle atrophy, visceral organ atrophy, and anergy. [13]

More recently, Roubenoff and colleagues[14] have proposed that the terms *wasting*, *cachexia*, and *sarcopenia* be considered as three distinctly defined entities ( Table 42-2). *Wasting* is defined as involuntary weight loss and is found in patients with anorexia nervosa, cancer, advanced human immunodeficiency virus infection, and marasmus. *Cachexia*, in contrast, is defined as involuntary loss of fat-free mass in the setting of minimal or no overall weight loss. This type of malnutrition can be seen in some patients with cancer, as well as in those with critical illness and early human immunodeficiency virus infection. In this scenario, patients of normal weight can still be malnourished due to reduced lean body mass. Because a decline in lean body mass has important functional and prognostic significance, even in the setting of stable or increasing weight, this type of malnutrition is important to identify. *Sarcopenia* refers to the involuntary loss of muscle mass that occurs with aging.

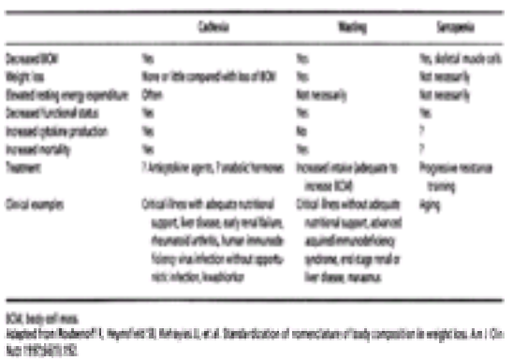

**TABLE 42-2. PARADIGMS OF WEIGHT LOSS/BODY COMPOSITION CHANGES IN ILLNESS**

The pattern of weight loss and changes in body composition in patients with illness are important to consider, because differential loss of body fat versus fat-free mass implies a different etiology and prognosis of malnutrition. For example, prolonged fasting in the absence of metabolic perturbation (as can be seen in adolescents with anorexia nervosa) leads to a predictable decrement first in body fat, then in body protein stores. [15] In these cases, energy repletion is usually successful with the provision of adequate energy, protein, and micronutrients.

In contrast, weight loss in the setting of cancer, infection, or other metabolic stress is composed of both fat and fat-free mass. [16] Because the energy density of fat-free mass is lower than that of fat, the body's use of lean body mass as an energy and amino acid source can lead to weight loss that can be quite profound and rapid. It is this loss of fat-free mass that is of significance, because loss of lean body mass is associated with important functional changes such as loss of strength, decreased immune function, decreased pulmonary function, increased disability, and death. [17,18,19,20 and 21]

More important, the provision of nutritional support to these patients may not be adequate to reverse the catabolic effects of the underlying condition. In the early days of parenteral nutrition (PN) use, it was hypothesized that aggressive PN could overcome the catabolism of cancer and other critical illnesses [22]; however, this has not proved to be the case. Instead, a more realistic appreciation for the limitations of nutritional support has emerged. [23] The utility and indications for PN in the pediatric cancer patient are reviewed in the following sections.

## EPIDEMIOLOGY

The occurrence of wasting among cancer patients is determined by host susceptibility, tumor type and location, and anticancer regimen. Because during infancy and adolescence there are increased energy needs for growth, children in these age groups are at increased risk of malnutrition. Other patients at higher risk of malnutrition are those with advanced disease and metastatic solid tumors, and those needing protracted chemotherapy ( Table 42-3). A striking example of malnutrition is the diencephalic syndrome in which a hypothalamic tumor presents with severe weight loss in the setting of a normal appetite. [24,25]

| |
|---|
| **High nutritional risk** |
| Advanced diseases during initial intense treatment |
| Unfavorable histology Wilms' tumor |
| Stages III and IV neuroblastoma, especially unfavorable biology |
| Advanced-stage rhabdomyosarcoma |
| Advanced-stage Ewing's sarcoma |
| Some non Hodgkin's lymphomas |
| Tumors of the head and neck (e.g., nasopharyngeal carcinomas) |
| Acute myelogenous leukemia |
| Some poor-prognosis acute lymphoblastic leukemias |
| Acute lymphoblastic leukemias during induction |
| Multiple relapse leukemia |
| Medulloblastoma and other high-grade brain tumors |
| **Low nutritional risk** |
| Good-prognosis acute lymphoblastic leukemia |
| Nonmetastatic solid tumors |
| Advanced diseases in remission during maintenance treatment |

Adapted from Mauer AM, Burgess JB, Donaldson SS, et al. Special nutritional needs of children with malignancies: a review. J Parenter Enteral Nutr 1990;14(3):315; and Rickard K, Grosfeld J, Coates T, et al. Advances in nutrition care of children with neoplastic diseases: a review of treatment, research, and application. J Am Diet Assoc 1986;86:1666.

**TABLE 42-3. RISK FACTORS FOR MALNUTRITION IN PEDIATRIC CANCER PATIENTS**

In contrast, most patients with low-risk acute lymphoblastic leukemia (ALL), those with nonmetastatic solid tumors, and patients in remission are generally able to maintain a normal weight. It should be noted, however, that weight loss may be considered the final step in a long process of nutritional perturbation in patients with cancer and, as such, may not be a sufficiently sensitive marker for malnutrition. For example, one study of prepubertal children with low-risk ALL followed body weight and lean body mass over time (as measured by sum of four skinfold measurements and bioelectrical impedance). Although body weight in the cancer patients was no different than age- and sex-matched controls, lean body mass declined substantially with the use of chemotherapy. [26] Thus, mere reliance on body weight to document normal nutritional status may lead to an underestimate of the prevalence of lean body mass depletion.

## ETIOLOGY AND PATHOPHYSIOLOGY

It is axiomatic that patients with weight loss exhibit reduced dietary intake, malabsorption or maldigestion of foods, or altered energy and nutrient needs. The challenge of nutritional care of pediatric cancer patients is that all three mechanisms may be at play, so that the clinician's diagnostic and therapeutic tools must be broadly considered.

In brief, weight loss or gain is due to energy imbalance, which ensues when energy intake differs from total energy expenditure (TEE). TEE, in turn, is considered to be the sum of several components of the energy equation. That is, in a steady state,

$$\text{Energy intake} = \text{TEE} = \text{REE} + E_{activity} + E_{growth} + E_{losses} + \text{SDA}$$

where

REE = resting energy expenditure (an estimate of basal metabolic rate),

$E_{activity}$ = energy needs for activity,

$E_{growth}$ = energy needs for normal growth,

$E_{losses}$ = energy needs for obligatory losses in urine and stool,

SDA = specific dynamic action of food, the energy needs for digestion and absorption

Weight loss occurs when any of the components of TEE are higher than expected and are not matched by a compensatory increase in energy intake. Weight gain occurs when energy intake exceeds TEE. Although an increase in energy intake is the most common reason for overweight, reduction in energy of activity has also been implicated in the development of obesity.[27]

### Decreased Nutrient Intake

Many studies have confirmed the clinical impression that cancer patients, especially when undergoing chemotherapy or radiotherapy, ingest lower amounts of nutrients than do age-based standards[28]; these dietary changes, therefore, place them at risk of negative energy balance and micronutrient deficiencies. For example, more than two-thirds of teenagers with ALL were found to be consuming less than 80% of the recommended dietary allowance for energy. [26]

There are multiple reasons for decreased nutrient intake. One is the well-known occurrence of circulating cytokines, including tumor necrosis factor alpha (formerly called cachectin), that induce anorexia. [11] The effects of proinflammatory cytokines have been studied as the etiology of multiple metabolic phenomena of cancer and its treatment and are more fully considered below.

Another important reason for decreased nutrient intake is anorexia and other gastrointestinal side effects of chemotherapy and radiotherapy. Mucosal damage is generally dose related, with increased risk of mucosal toxicity with high-dose induction therapy, escalating dosage patterns, continuous infusion (versus bolus doses),

and combination chemotherapy treatments. High-dose chemotherapy and total body irradiation as conditioning for bone marrow transplantation (BMT) often produce painful oral mucositis that can reduce nutritional intake for days to weeks. Other gastrointestinal side effects of cancer treatment include esophagitis and enteritis with malabsorption and diarrhea; these are more fully described in Chapter 39 and Chapter 44. Taste perception has also been shown to be altered in cancer patients receiving chemotherapy, with an increasing sensitivity to bitter tastes; this phenomenon may lead to reduced food intake, and may make the use of oral supplements difficult.

Psychological factors are also important to consider in evaluating the reasons behind inadequate dietary intake. Depression-related anorexia is probably underappreciated as a cause. Appetite and feeding behaviors are inherently complicated activities of all children, and this behavior can obviously be affected by the onset of illness and its treatment, as well as the psychosocial impact that cancer has on the child and family. The nature of cancer medical care is such that parents often feel that they are relinquishing much of their usual caregiving behavior to the medical and nursing staff. Some parents understandably cling to the provision of food and nutrients as a critical part of parenting; indeed, the terms *nutrition*, *nursing*, and *nurture* all share the same Latin root. Occasionally, such strong feelings concerning food and dietary intake can cause conflict between parent and child and be counterproductive to the goal of optimal nutrition.

### Changes in Energy Expenditure: Resting Energy Expenditure

Although decreased nutrient intake is common in cancer, anorexia alone cannot wholly explain the common development of malnutrition because some patients maintain an excellent intake but still suffer progressive weight loss. Therefore, it is not surprising that much research has focused on the possible role of increased energy expenditure of cancer patients in trying to explain cancer cachexia. Unfortunately, although studies have been performed for more than 50 years on this topic,[29,30] and [31] the issue has not been consistently resolved.

In brief, earlier studies suggested that the energy needs of rapidly dividing cells of the tumor increased the basal metabolic demands of the host from 20% to 90% over predicted needs. Because basal energy needs account for a substantial portion of total energy needs, any increment in basal energy requirements could clearly result in energy imbalance. More recent data have confirmed that hypermetabolism can occur but not in all patients at all times. In a large cohort study, Knox and colleagues[32] studied 200 adult cancer patients using the technique of indirect calorimetry, a noninvasive bedside measure of REE, the clinical estimate of basal metabolic rate.[33] Their subjects had solid organ malignancies, such as tumors of the gastrointestinal, gynecologic, or genitourinary tracts. They found that one-third of patients was hypometabolic (REE was less than 90% of predicted levels), one-fourth was hypermetabolic (REE greater than 110% predicted), and the remaining 40% had normal REE (between 90% and 110% predicted). Older subjects, those with longer duration of disease, and underweight patients tended to have higher REE measurements. Others have reported that tumor type may play a role in effecting energy expenditure. Patients with lung cancer have been reported to have an increased REE, but those with gastric and colon cancer showed no change. [34]

In children, there have been fewer studies of this issue. An infant with diencephalic syndrome was found to have a strikingly increased TEE, as measured by the doubly labeled water technique.[35] Stallings and colleagues[36] measured REE in nine patients with ALL and found that patients with a higher tumor burden (elevated white blood cell count, organomegaly) had an increased REE. A study of 26 patients with ALL or solid tumors in remission showed no evidence of an increased REE when compared to age- and sex-matched healthy controls.[37]

### Changes in Energy Expenditure: Energy of Activity

As noted above, total energy requirements include energy needed for physical activity. Recent studies have shed light on this part of the energy equation in pediatric cancer patients. An interesting population of children with cancer whose energy balance has been studied are those with ALL. Although generally well nourished on presentation, some children with ALL actually become obese.[38] Reilly and colleagues[39] studied 20 preadolescent children with ALL with doubly labeled water, a technique which reliably measures TEE in free-living individuals. [40] Compared to healthy controls matched for age, sex, and body composition, children with ALL had significantly lower TEE than did controls by an average of 282 kcal per day. Most of this reduction was due to reduced physical activity of the patients. A similar study, using a combination of indirect calorimetry and ambulatory heart rate monitoring to measure basal metabolic rate and TEE, also concluded that ALL patients have lower levels of energy expenditure for activity. [41] The implications of these findings for obesity prevention in ALL survivors, as well as children at large, may be significant.

## ALTERATIONS IN MACRONUTRIENT METABOLISM

Children with cancer manifest changes in carbohydrate, lipid, and protein metabolism. The pattern of macronutrient use in malignancy is markedly different than that evident with starvation. The tumor itself seems to impose a pattern of perturbations that lead to catabolism; however, the extent of this response is variable. This may be due to the heterogeneity of tumor types and sizes, variant treatment protocols, and differences in baseline nutritional status. A general understanding of these alterations is, however, useful in anticipating potential metabolic complications as well as planning nutritional therapy.

### Carbohydrate

The changes seen in carbohydrate metabolism that are associated with malignancy generally consist of glucose intolerance, increased gluconeogenesis (the conversion of amino acid carbon skeletons to glucose),[42] and increased Cori cycling (the hepatic conversion of lactate to glucose). [43] Glucose intolerance in cancer patients is at least partially due to the presence of insulin resistance. [44,45] and [46] This has been demonstrated with various tumors by documenting decreased glucose uptake under steady states of hyperinsulinemia induced during glucose clamp studies. The augmented conversion of lactate to glucose may be secondary to increased lactate production by selected tumors. Overall, the enhanced production of glucose provides the tumor with a substrate that is readily metabolized under both aerobic and anaerobic conditions.

### Lipid

Lipid metabolism is also affected by cancer. Alterations include increases in free fatty acid turnover, free fatty acid oxidation, glycerol turnover, and lipolysis. Lipogenesis is reduced. A lipid mobilizing factor has been isolated from the urine of cachectic cancer patients and shows bioactivity with isolated murine adipocytes. [47] As may be expected, these changes are accompanied by a marked loss of body fat that can occur early in the evolution of the malignancy. [48] Children with ALL and widespread solid tumors tend to have elevated triglycerides as do children in remission treated with L-asparaginase.[49] The provision of glucose does not seem to resolve the high rates of fatty acid and glycerol turnover evident in weight-losing cancer patients. [50] Treatment with chemotherapy is associated with decreased fat use in children newly diagnosed with ALL.[36]

### Protein

Another salient derangement of macronutrient metabolism accompanying cancer is the presence of protein catabolism. Hypoalbuminemia is common, whereas the synthesis of acute phase proteins remains high.[51] Some tumors, such as hepatocellular cancer, manifest very high rates of protein turnover and increased protein degradation.[52] An increase in muscle protein breakdown mobilizes amino acids that may afford tumor growth as well as fuel gluconeogenesis. This increased protein breakdown in pediatric cancer patients may be related to falling levels of insulin-like growth factor and insulin-like growth factor binding proteins. [53] In other patients, decreased skeletal muscle protein synthesis seems to be of primary importance.[54] Although the mechanism remains uncertain, a net loss of skeletal muscle protein is a common finding with malignancy and is particularly problematic in the growing child.

### Cytokines

Proinflammatory cytokines commonly associated with cancer cachexia include tumor necrosis factor a (TNF-a), interleukin (IL)-6, IL-1, and interferon g. These cytokines are secreted by macrophages and lymphocytes and may represent the host response to cancer. TNF-a was first identified as a mediator of cachexia in an animal model of infection.[55] In humans, the acute administration of TNF-a produces effects associated with cancer cachexia, including increased fatty acid turnover, elevated glycerol turnover, and increased whole-body protein turnover. [56] Not all patients with cancer cachexia have detectable levels of TNF-a; hence, a paracrine or autocrine mechanism of action may be responsible. Other factors also seem to be involved with the catabolic response to cancer. Circulating IL-6 levels are generally elevated in cancer patients and transfection of the IL-6 gene into nude mice does engender cachexia. [57] As with TNF-a, the precise mechanism of action of IL-6 is unknown. The metabolic roles of IL-1 and interferon g are less well characterized in tumor models; however, as with TNF-a and IL-6, they appear to inhibit lipoprotein

lipase and hence facilitate lipolysis. Various medications with cytokine inhibitory properties, such as pentoxifylline and megestrol acetate, are being evaluated as possible therapies for cancer cachexia.[58]

## CLINICAL ASSESSMENT OF NUTRITIONAL STATUS

The assessment of a child with cancer includes standard elements of a nutritional evaluation: history of past and present illness, review of dietary intake, physical examination, and anthropometric measurements. Attention should also be directed to the significance of body composition changes, as well as a review of pertinent laboratory measures.

### History

A detailed medical history is essential to the nutritional evaluation of a patient. Perhaps the most important historical aspects are the type and stage of the tumor, the intensity of the planned antitumor therapy, and the presence or absence of remission. As noted above, there are important risk factors for the development of malnutrition in cancer patients (Table 42-3).[8]

Nutritional history should include (a) elicitation of current symptoms of cancer and its therapy and their effect on nutrient intake, absorption, and retention; (b) past history, including past growth data; previous antitumor therapy, and its effects on nutritional status; (c) developmental status, with special attention to milestones of feeding skills and swallowing function; (d) known or perceived food allergies or intolerances; (e) medications, with special attention to those with gastrointestinal side effects (Table 42-4); (f) family history, parental heights, and sibling growth patterns; and (g) social history, food preferences/beliefs, and food availability.

| Category | Examples | Side effects |
|---|---|---|
| Chemotherapy | Busulfan | N&V, mucositis, hepatic (high dose) |
| | Cyclophosphamide | N&V, altered tastesmell |
| | Cytarabine | N&V, mucositis, diarrhea |
| | Dactinomycin | N&V, mucositis, hepatic, diarrhea |
| | Doxorubicin | Severe mucositis, N&V, diarrhea |
| | L-Asparaginase | Pancreatitis, hepatic, hyperglycemia, altered tastesmell |
| | Melphalan | N&V, mucositis, diarrhea |
| | Methotrexate | Mucositis, diarrhea, hepatic |
| | Vincristine | Neurotoxicityileus |
| Corticosteroids | Prednisone, dexamethasone | Hyperglycemia, fluid and salt retention, muscle wasting |
| Antibiotics | Augmentin, erythromycin, clindamycin | Diarrheastomach cramps |
| Narcotics | Codeine, morphine | Ileus |
| Radiation | Total body irradiation, focal irradiation | Mucositis, radiation enteritis (depending on site) |

N&V, nausea and vomiting.
Adapted from Bechard L. Oncology and bone marrow transplantation. In: Hendricks K, Duggan C, Walker W, eds. Manual of pediatric nutrition. Hamilton, Ontario: B.C. Decker; 2000.

**TABLE 42-4. GASTROINTESTINAL SIDE EFFECTS OF TREATMENTS USED IN PEDIATRIC ONCOLOGY**

The ability of children to eat at their appropriate developmental level may affect the nutritional adequacy of their diet. Anorexia, mucositis, and other effects of cancer treatment may interrupt the normal progression of feeding skills in infants and young children. Once arrested, the development of these skills may be difficult to restore. Poor swallowing and chewing abilities lengthen the time required to complete a meal and thus may lead to inadequate consumption of many required nutrients. Children may refuse to eat their preferred foods due to adverse associations or other impairments and thus may self-restrict their intake.

A thorough diet history obtained from the patient or caregivers may be analyzed to detail the nutritional intake of the child with cancer. A 24-hour dietary recall, which is the most rapid dietary intake method, can be easily incorporated into the general history and physical examination. Problems of recall bias, and under- or overreporting of intake have been noted,[59] however.

A prospective food diary, in which a subject measures and writes down all intake for 3 to 7 days, may be the most reliable and valid clinical tool for evaluating nutritional intake. Proper interpretation of these diet records requires consultation with a qualified dietitian. The nutritional composition of these foods is often determined by using one of the common nutritional databases, often with the use of proprietary software.[60] An average daily intake of energy, macronutrients, and micronutrients can then be calculated and compared with published reference data. Prospective diet records should generally be performed while the patient is feeling well, free of the effects of acute illness, and should include at least one weekend day in school-age children.

### Physical Examination

The physical characteristics of a child with cancer may suggest the presence of nutritional problems. Indeed, the predisposition of chemotherapy to affect tissues of rapid turnover (e.g., hair and gastrointestinal mucosa) mirrors the symptoms of deficiency of a wide range of nutrients (e.g., stomatitis with some B vitamins, alopecia with biotin deficiency). Careful inspection and palpation of subcutaneous fat and muscle stores are particularly important in the physical examination, because deficits of these two components of body stores are common in malnutrition.

The general physical examination may show edema as a result of low concentrations of circulating albumin. This can be a subtle finding with only mild hypoalbuminemia. Edema may actually mask progressive muscle wasting and loss of lean body mass. Hypoalbuminemia may be due to poor liver function related to tumor or treatment, inadequate protein intake, or nutrient losses in excess of intake.[61] Painful mouth or esophageal sores caused by mucositis can negatively impact a child's oral intake and thus be an impetus to consider nutritional support.[62] Alopecia and stomatitis, commonly associated with chemotherapy, can also be caused by vitamin or mineral deficiencies.[63] Children who have limited activity due to impairment by disease or treatment side effects may experience changes in appetite or satiety. Recognition of significant changes in the physical examination is a valuable clue to the overall nutritional assessment of children with cancer.

### Anthropometrics

Weight and height measured over time are the mainstays of nutritional monitoring and evaluation.[64] When compared to age- and sex-appropriate standards, these data can provide objective measures of nutritional status and provide the most information for the least inconvenience and cost. Indications for intensified nutritional assessment and monitoring based on weight and height measures are listed in Table 42-5. Weight should be measured daily in the hospital setting and at each office visit for outpatients. Weight of infants should be recorded to the nearest gram with the child wearing no clothes or a diaper. In older children, shoes and heavy clothing should not be worn, and the scale should be accurate to the nearest 0.1 kg. Length should be measured on a length board with a tape measure attached and a moveable footboard. Children older than 2 years should be measured with a stadiometer while standing erect. Height/length measurements should be recorded to the nearest 0.1 cm. Accurate technique is critical to the validity of the measures; detailed methodologies are outlined elsewhere.[65,66] Length should be measured every 1 to 3 months in infants, and at least yearly for older patients. Measurements should be plotted on the National Center for Health Statistics (NCHS) growth curve and compared to established norms for age and sex, and the corresponding percentile should be determined. Updated reference curves have recently been published by the NCHS of the Centers for Disease Control and Prevention at http://www.cdc.gov/growthcharts/.

| Criteria | Risk |
|---|---|
| Height for age below tenth percentile | Chronic malnutrition |
| Height velocity <5 cm/yr after 2 yr of age | |
| Weight for height (<10 yr) below tenth percentile | Acute malnutrition |
| Weight velocity <1 kg/yr (prepubertal) | |
| Weight for age (>10 yr) below tenth percentile | |
| Weight velocity (peak) <1 kg/6 mo (pubertal) | |
| Weight for height (<10 yr) above ninetieth percentile | Obesity |
| Weight for age (>10 yr) above ninetieth percentile | |

Adapted from Motil KJ. Sensitive measures of nutritional status in children in hospital and in the field. Int J Cancer 1998;11(Suppl):2.

**TABLE 42-5. INDICATIONS FOR NUTRITIONAL ASSESSMENT**

The most widely used anthropometric screening criteria for malnutrition in children are those of Waterlow ( Table 42-6).[67] These criteria recognize that children who are underweight may be either short and well proportioned (so-called stunted) or truly underweight for their height (so-called wasted). Wasted children have an acute deficit of body mass and have a variety of functional deficits (e.g., decreased muscle strength, impaired immune function, and decreased organ mass). The incidence of weight and height deficits in children with cancer has been reported to be quite high, depending on the severity and type of cancer and its treatment. [8]

| | Degree of malnutrition | | | |
|---|---|---|---|---|
| Criteria | 0 (normal) | 1 (mild) | 2 (moderate) | 3 (severe) |
| Weight-for-height (% expected) | 90–110 | 80–90 | 70–80 | <70 |
| Height-for-age (% expected) | ≥95 | 90–95 | 85–90 | <85 |
| | Degree of obesity | | | |
| | 1 (overweight) | 2 (obese) | 3 (morbid) | |
| Weight-for-height (% expected) | 110–120 | 120–140 | >140 | |

Adapted from Metil KJ. Sensitive measures of nutritional status in children in hospital and in the field. Int J Cancer 1998;11(Suppl):2; and Waterlow J. Classification and definition of protein-calorie malnutrition. BMJ 1972;3:566.

**TABLE 42-6. ANTHROPOMETRIC CRITERIA FOR THE DIAGNOSIS OF MALNUTRITION AND OBESITY IN PEDIATRIC CANCER PATIENTS**

Due to the increased incidence of obesity in children in industrialized countries [68] and the predisposition of some pediatric cancer patients to become obese, it is equally important to screen for overweight. This can be done by calculating the weight-for-height percent standard. An increasingly used anthropometric index is body mass index (BMI),[69] which is a reasonable measure of body fatness. Children with BMIs greater than the eighty-fifth percentile for age and sex are termed *overweight*, and those with BMIs greater than the ninety-fifth percentile are obese. Reference values for BMIs are available [70] and are included in the 2000 NCHS growth curves.

### Arm Anthropometrics

Arm anthropometrics are an inexpensive, noninvasive, and widely accepted technique for estimating body composition against established normal values. Mid-arm circumference is measured with a nonstretchable flexible tape at the midpoint between the acromion and the olecranon with the arm flexed at a 90-degree angle. Triceps skinfold is measured on the back of the arm at this same point with a caliper exerting a constant pressure of 10 g per $mm^2$. With these two measurements, the mid-arm muscle area can be calculated:

$$\text{MAMA} = \left(\frac{\text{MUAC} - \text{BTSF}}{4\pi}\right)^2$$

where

MAMA = mid-arm muscle area ($cm^2$),

MUAC = mid–upper arm circumference (cm),

TSF = triceps skinfold (cm),

p = 3.1416

Percentiles and standards for comparisons for triceps skinfolds and mid-arm muscle area are available [71] and may indicate the presence of malnutrition when weight or height is unavailable or invalid.

A study of 16 boys with newly diagnosed cancer revealed no significant deviation from normal with respect to weight, height, and weight for height. When comparing the mid-arm circumference, triceps skinfold, and subscapular skinfold to normal values, however, significant reductions were found in the patients with cancer. [72] This and other studies suggest that arm anthropometrics may be more sensitive indicators of undernutrition than are weight and height.

### Measures of Body Composition

Anthropometric measurements may be confounded by technique, inaccuracy of equipment, lack of patient cooperation, and discrepancies between weight and protein energy reserves. Lean body mass is the metabolically active component of the body. It would be ideal, therefore, to characterize weight by some measure of lean body mass to accurately assess the need for or response to nutritional intervention. [72] Patients with solid tumors before reduction or excision are particularly susceptible to inaccurate weight measurements. In a study of 19 children with malignant solid tumors, regional ultrasonography of the femoral quadriceps muscle was more sensitive than anthropometrics and measurements of visceral proteins in detecting reduced muscle protein reserves compared to age- and sex-matched controls,[73] suggesting the need for further analysis of body composition in assessing nutritional status.

Bioelectrical impedance is an inexpensive and increasingly available method of assessing body composition. The technique relies on the principle that fat-free mass, being composed of water and ions, conducts an electrical charge faster than does fat mass. [74] Lean body mass, therefore, has a lower resistance to current. Reactance, a measure of cell membrane capacitance, is also measured by bioelectrical impedance and together with resistance can be used to measure total body water, both intra- and extracellular. Fat-free mass is calculated using assumptions about its water content.

Dual-energy X-ray absorptiometry (DEXA) is becoming increasingly more available for clinical use. DEXA is able to measure lean body mass, fat mass, and bone mass, thereby accounting for any abnormalities in bone-mineral density. [75] Specifically, DEXA scanning determines bone mineral content, bone mineral density, and total body bone mineral content. Some instruments assess total body bone mineral content, nonbone lean tissue, and fat, thereby providing body composition information using a three-compartment model. A number of studies have compared measurements of fat mass with DEXA versus results obtained from underwater weighing (hydrodensitometry),[76] and the correlations between the methods have generally been high. Unlike hydrodensitometry, DEXA also measures the composition of particular body parts, thus allowing one to compare visceral and subcutaneous adiposity. The ultimate applicability of DEXA scanning to human body composition and nutritional assessment is still evolving. [77]

### Laboratory Evaluation

Although laboratory evaluation of the pediatric cancer patient is commonly performed to assess the hematologic and metabolic response to cancer and its treatment, the clinician should also monitor nutritional status with selected biochemical parameters. Serum electrolytes and mineral concentrations are commonly deranged during treatment of cancer, especially when nephrotoxic agents are used. A specific metabolic emergency termed *tumor lysis syndrome* occurs when the tumor mass is suddenly reduced with initial chemotherapy. The massive cell lysis can lead to life-threatening electrolyte abnormalities, as discussed in detail in Chapter 39.

Biochemical parameters used for nutritional assessment include visceral protein levels. These proteins synthesized by the liver are often used to assess nutritional status, because decreased levels presumably reflect a reduced supply of amino acid precursors or decreased hepatic and other visceral protein mass. Blood concentrations of these proteins, however, are dependent on their rates of synthesis, degradation, and escape from the circulatory system. Serum proteins are also affected by infectious or catabolic processes. The concentrations of positive acute phase proteins (e.g., C reactive protein, ferritin, ceruloplasmin) are increased in infectious or other catabolic illnesses, whereas negative acute phase proteins (e.g., albumin, prealbumin, transferrin, and retinol binding protein) are decreased in these circumstances.

Albumin is the most abundant serum protein, making up nearly 5 of the 10 grams per deciliter of total protein in the serum. It is the least expensive and easiest protein to measure, and therefore is the most commonly used biochemical marker to assess protein status. Because more than half of body albumin is extravascular (primarily in skin and muscle), maintenance of normal serum levels can occur from mobilization of these stores despite prolonged energy or protein inadequacy. Combined with a long half-life of 20 days, these factors make serum albumin a relatively insensitive marker of nutritional status. Hypoalbuminemia is not necessarily diagnostic of malnutrition; it can occur in situations of decreased synthesis (e.g., liver disease, age older than 70 years, malignancy), increased losses (e.g., nephrosis, protein-losing enteropathy, burn injuries), or increased losses to extravascular spaces (e.g., acute catabolic stress with capillary leak syndrome). Fluid overload can also dilute albumin concentrations, and bed rest can decrease levels by 0.5 g per dL.

Prealbumin is another visceral protein, named because of its proximity to albumin on an electrophoretic strip. Prealbumin circulates in plasma in a 1:1 ratio with retinol-binding protein. Its short half-life (2 days) and high ratio of essential to nonessential amino acids make it a good measure of visceral protein status, more sensitive than albumin as a measure of nutritional recovery. Studies have shown prealbumin to correlate well with nitrogen balance, [78,79] and it is likely the best available serum marker of nutritional status. Similar to albumin, concentrations fall with an acute phase protein response or liver disease. Levels increase with renal failure.

In a study of 170 children with an assortment of cancers, albumin and prealbumin levels were significantly lower on presentation in patients than in controls, whereas the acute phase indicator C-reactive protein was elevated. After 6 months of chemotherapy, however, the C-reactive protein levels were comparable in the groups but the visceral protein levels were still lower in those with cancer. [80] This suggests that even though the acute phase response is responsible for some alterations in visceral protein levels, cancer patients still have poorer visceral protein nutrition than do controls.

It may also be helpful to assess vitamin and mineral nutriture, particularly in the patient on total enteral or parenteral nutritional support. At the time of diagnosis, the effect of tumor burden on metabolic processes may be profound, as demonstrated by laboratory analysis. In a study of 40 children with ALL, more than 70% had low plasma 1,25-dihydroxy vitamin D levels, 73% had low osteocalcin levels, and 64% had hypercalciuria at the time of diagnosis. This is an indication of the effect of leukemia on vitamin D metabolism and bone turnover. The use of steroids and nephrotoxic agents may worsen hypercalciuria, putting patients at a greater risk for osteopenia fractures. Excessive renal losses of magnesium during treatment with these medications were also demonstrated, with only 50% of patients able to maintain normal serum magnesium levels despite parenteral supplementation. [81]

Antioxidant nutrients, including vitamins A and E, beta-carotene, zinc, and selenium have also been studied. Children with a variety of cancers had lower concentrations of retinol, beta-carotene, vitamin E, and zinc before treatment compared to controls but were not significantly different than controls after treatment. When separated by diagnosis, however, zinc concentrations in patients with central nervous system tumors and malignant bone tumors were persistently lower after 6 months of treatment. [80]

Zinc deficiency was also found to be common in children after BMT. Nineteen of twenty-eight children developed biochemical zinc deficiency at a median of 7 ays posttransplant. Zinc blood levels were positively correlated with serum concentrations of alkaline phosphatase, a zinc-dependent enzyme. [82]

## NUTRITION INTERVENTION TECHNIQUES

The method of nutrition support chosen is based on the clinical assessment and the child's nutrient requirements. [83] Some children may require minor alterations to their oral diet; others may require specialized enteral or parenteral support. There is increasing evidence that enteral support is a less expensive, safer, and effective way of nourishing the child with cancer than PN. [84,85] Patients and their family members should, of course, be included in the discussion of nutrition options, with accurate depiction of the risks and benefits of the possible methods of nutrition support given the patient's current situation.

When treating the malnourished child, it is imperative to consider the manifestations of refeeding the body in a starved state. A profound hypophosphatemia, in particular, can characterize the rapid cellular influx of nutrients during the anabolic phase following starvation. Severe metabolic complications may be avoided by the slow advancement of macronutrients over a period of days to weeks, as well as close laboratory monitoring of electrolytes, glucose, and minerals.

### Oral Feeding

Modifications to the oral diet for pediatric oncology patients include reduced bacteria, [86] texture changes, adjustments to electrolyte or mineral content, and calorie supplementation. Although the efficacy of low-bacteria diets to reduce infections has not been proved, some centers continue to use them routinely, particularly in the BMT setting. General principles of cautious food safety should be followed for any immunocompromised child ( Table 42-7).

| |
|---|
| Wash hands well before and after preparing and eating meals. |
| Do not share food with others. |
| Avoid foods from street vendors, salad bars, and shared bins of foods in grocery stores. |
| Wash raw foods well before eating. |
| Cook meat until well done. |
| Avoid raw eggs. |
| Keep foods at temperatures <40°F or >140°F to minimize growth of bacteria. |
| Clean all preparation items thoroughly before and after use to avoid cross-contamination. |
| Keep refrigerated leftovers for no more than 3 d. |

Adapted from Bechard L. Oncology and bone marrow transplantation. In: Hendricks K, Duggan C, Walker W, eds. Manual of pediatric nutrition. Hamilton, Ontario: B.C. Decker, 2000.

**TABLE 42-7. SANITARY FOOD PRACTICES FOR IMMUNOCOMPROMISED PATIENTS**

Children with mucositis may better tolerate a soft diet. A diet high in magnesium or potassium may be useful for the child with excessive urinary losses of minerals due to chemotherapies or antibiotics. Most commonly, however, calorie supplementation is required to assist with weight gain or weight maintenance during cancer treatment. This can be accomplished with usual foods or in combination with commercial supplemental drinks, bars, or calorie additives. Techniques for increasing oral energy intake are listed in Table 42-8.

| |
|---|
| Loss of appetite |
| Offer small frequent feedings (6-8 meals or snacks per day). |
| Encourage nutrient-dense beverages between meals. |
| Offer favorite nutritious foods during treatment-free periods to prevent learned food aversions. |
| Nausea and vomiting |
| Feed 3 to 4 h before therapy that typically causes nausea and vomiting. |
| Offer small amounts of cool foods and encourage slow eating; avoid strong odors. |
| Offer clear liquids between meals; using a straw in a covered cup may facilitate sipping. |
| Mouth sores |
| Serve soft or pureed bland food or liquids. |
| Add butter, gravy, sauce, or salad dressing to moisten foods. |
| Avoid highly seasoned or hard, rough foods. |
| Altered taste perception |
| Use stronger seasonings; avoid excessively sweet foods. |
| Offer salty foods (e.g., hot dogs, pizza, canned pasta). |
| Try new flavors of foods. |

Adapted from Bechard L. Oncology and bone marrow transplantation. In: Hendricks K, Duggan C, Walker W, eds. Manual of pediatric nutrition. Hamilton, Ontario: B.C. Decker, 2000.

**TABLE 42-8. STRATEGIES FOR IMPROVING ORAL INTAKE DURING CANCER TREATMENT**

Some children, particularly long-term survivors of cancer with activity limitations, may be aided by the instruction of moderate-calorie and low-fat diet principles when obesity is a concern (Table 42-9).

| |
|---|
| Aim for fitness |
| Aspire to a healthy weight. |
| Be active each day within personal limitations. |
| Build a healthy base |
| Follow the eating guidelines of the food guide pyramid. |
| Eat a variety of whole grains, fruits, and vegetables each day. |
| Choose sensibly |
| Select a diet low in saturated fat and cholesterol. |
| Keep total fat intake <30% of calories. |
| Limit sugary foods and beverages. |
| Choose and prepare foods with less salt. |

Adapted from Excerpt of the Report of the Dietary Guidelines Advisory Committee on Dietary Guidelines for Americans. Washington, D.C.: United States Department of Agriculture, 2000. http://www.ars.usda.gov/dgac/.

**TABLE 42-9. HEALTHY EATING GUIDELINES FOR CHILDHOOD CANCER SURVIVORS**

## Enteral Feeding

If children are unable to meet their nutrient needs orally, tube feedings should be considered as a means of preserving or obtaining optimal nutritional status. Nasogastric tubes have been used with success in many pediatric oncology patients. In 21 children newly diagnosed with cancer, nasogastric tube feedings were given in addition to volitional oral intake. After 16 weeks of feedings, weights increased significantly. [87] Another study using nasogastric feedings in children after BMT demonstrated improved weight gain in 21 patients, compared to eight children receiving dietary counseling alone. There was a positive correlation between increases in weight and mid-arm circumference and the duration of enteral feedings. However, eight of the 21 patients stopped feedings after 10 days due to vomiting or diarrhea. Six of these patients were then switched to PN support.[88] These studies suggest the feasibility of nasogastric feeding as a method of nutrition support for pediatric cancer patients, although clearly some patients will be unable to tolerate the required volumes to support their nutritional requirements, particularly in the BMT setting.

Gastrostomy tube feedings are generally considered to be more cosmetically acceptable and comfortable than nasogastric feedings, while still providing the same advantages over PN. The use of surgically placed or percutaneous endoscopic gastrostomy feedings in pediatric cancer patients has been examined by several groups (Table 42-10).

| Reference | Number of patients | Type of tube | Complications | Weight changes |
|---|---|---|---|---|
| [illegible] | [illegible] | PEG | No major; 77% of patients experienced minor complications, including leakage of gastric juice and superficial wound infections | +2.3 standard deviation score of weight for age |
| [illegible] | [illegible] | 12 PEG, 2 surgical | 1 systemic infection; 9% of patients experienced minor complications, including skin inflammation, local infection, bleeding, and feeding intolerance | 82% achieved or maintained ideal body weight |
| [illegible] | [illegible] | PEG, surgical | 1.53 episodes of infections per 1,000 days of use compared with 5.0 episodes per 1,000 days for total parenteral nutrition | All gained or maintained weight |

PEG, percutaneous endoscopic gastrostomy tube

**TABLE 42-10. GASTROSTOMY FEEDINGS IN CHILDREN WITH CANCER**

The use of tube feedings for pediatric cancer patients can initiate weight gain and improved nutritional status with limited risks. Although some patients may ultimately require parenteral support due to gastrointestinal intolerance, the use of enteral nutrition substantially reduces the amount and duration of PN required, thus reducing the risks and costs with which it is associated.[84,85,89,90]

Nutritionally complete formulas for oral supplementation or tube feeding are available for a variety of ages and conditions. Most pediatric cancer patients will tolerate intact protein, 1-kcal-per-mL formulas, either orally or via tube feeding ( Table 42-11). On rare occasions, a specialized formula for tube feeding may be indicated. Formulas with an elemental (free amino acids) or semi-elemental (small peptides) base are available, primarily for the purpose of protein allergy or intolerance. Medium-chain triglycerides are also used in many formulas intended for patients with fat malabsorption. An abundance of defined formula diets are available for varying conditions and ages.

| Formula | Manufacturer | Flavors | Designed for ages |
|---|---|---|---|
| Boost | Mead Johnson Nutritionals | Chocolate, chocolate mocha, chocolate raspberry, vanilla, strawberry, strawberry banana | Teens and adults |
| Ensure | Ross Products | Vanilla, chocolate, strawberry, butter pecan, coffee | Teens and adults |
| Nutrabics | Nestle | Vanilla, chocolate, strawberry | Teens and adults |
| Ordercal | Mead Johnson Nutritionals | Vanilla | Children aged 1–10 yr |
| Pedisure | Ross Products | Vanilla, chocolate, strawberry, banana cream | Children aged 1–10 yr |
| Nutren Junior | Nestle | Vanilla | Children aged 1–10 yr |
| Resource Just for Kids | Novartis | Vanilla, chocolate, strawberry | Children aged 1–10 yr |

Note: Review manufacturer information.

**TABLE 42-11. INTACT PROTEIN FORMULAS FOR ORAL OR TUBE FEEDING**

## Parenteral Feeding

When the gastrointestinal tract is nonfunctional or unavailable, nutrients may be infused via central venous catheters. Peripheral venous lines may also be used temporarily to provide a nutrient solution with less than 900 mOsm per L, usually 10% dextrose and 1.5% to 2% amino acids. PN has been widely used in the oncology population due to the cytotoxic effects of many treatment regimens. Most chemotherapy regimens commonly cause some degree of nausea and vomiting. Radiation therapy, when directed to gastrointestinal organs, also causes cell damage that may impair digestive or absorptive function. High-dose chemotherapeutic regimens and total body irradiation, used in preparation for BMT, may cause a severe mucositis and enteritis, making significant oral or enteral intake difficult for many patients to achieve for several weeks.

The value of PN in cancer patients has been questioned; in fact, its routine use in adults undergoing chemotherapy has been discouraged due to the risks of infectious complications.[91] BMT, however, is one of the few clinical situations in which PN has demonstrated benefit. In a study of 22 patients receiving prophylactic PN compared to age- and diagnosis-matched historical controls, engraftment occurred significantly sooner in patients receiving prophylactic PN; there was no significant difference in overall clinical outcome. [92] In a subsequent prospective trial, patients were randomized to receive either PN or a 5% dextrose solution with additives for 1 week before and 4 weeks after transplantation. Although engraftment was not different among the two groups, overall survival, time to relapse, and disease-free survival were significantly better in the group receiving PN. [93] This study was instrumental in making PN an integral part of supportive care to BMT patients.

Since the publication of these studies, however, and with the exception of studies of glutamine (Gln) supplemented PN (see the section Glutamine and Other Experimental Nutrients in Pediatric Cancer), there have been relatively few well-designed trials to further advance the science of nutrition support in cancer patients. For instance, one group evaluated the effect of PN on visceral protein levels in children undergoing BMT and reported that concentrations of albumin and prealbumin rose significantly from day 0 until the end of PN administration. However, all subjects received the same PN regimen and no control data were presented. [94]

Some published studies in adults may shed light on the role of PN in the BMT setting, with the caveat that the significance of these data in children is difficult to assess due to the substantial differences in physiology and metabolism between adults and children. A study of adults with acute myelogenous leukemia examined the need for PN based upon three indications: severe malnutrition, a 7- to 10-day period of minimal oral intake, or weight loss of more than 10%. Patients receiving a mismatched BMT had the greatest incidence of PN use (92%), whereas only 35% of patients needed PN during consolidation courses. The authors concluded that PN was not required for all patients undergoing intensive cytotoxic therapy. [95] A study of 28 adults undergoing BMT compared the effects of a high nitrogen (330 ± 60 mg N per kg per d) PN solution versus a standard nitrogen (267 ± 44 mg N per kg per d) PN solution. [96] Caloric intake was kept constant. With the exception of nitrogen balance (better in the high-nitrogen-intake group), no clinical or metabolic differences were observed. This study underlines that fact that the severe catabolism of BMT conditioning cannot be reversed simply by providing higher protein intake.

In contrast, an interesting study of 15 adults evaluated the impact of reducing parenteral energy and protein intake after BMT. [97] Ten consecutive patients received 150% of estimated basal energy expenditure and 1.4 grams of protein per kg body weight and five subsequent patients received only 100% of estimated basal energy expenditure and 0.8 grams protein per kg body weight. No oral intake was allowed during the study; lipid emulsion was infused twice weekly to prevent essential fatty acid deficiency. The group receiving reduced amounts of energy and protein had significantly higher serum albumin levels, less hyponatremia, and less hyperkalemia compared to the group receiving higher amounts of energy. Nitrogen balance was similar in both groups. The results suggest the possibility of more efficient nitrogen utilization with a decrease in calories and protein, as well as improved metabolic homeostasis; further investigation of these results is warranted.

The use of lipids in PN has been questioned with regard to infectious risk and immunosuppressive effects. Clinical and metabolic effects of a lipid-based PN (80% nonprotein calories) versus a glucose-based PN (lipid free) were analyzed and suggested a lower incidence of lethal acute graft-versus-host disease and hyperglycemia in the lipid-based PN group. No significant differences in survival, time to engraftment, incidence of infections, or rate of relapse were seen. [98] This implies the possibility that intravenous (i.v.) lipids, due to their immunosuppressive effects, may have an effect on the severity of graft-versus-host disease. More research, particularly in children, is warranted.

When indicated, PN in children should be initiated according to the guidelines shown in Table 42-12. Goals of parenteral nutrients will be based on estimated requirements for age and nutritional assessment. Fluid requirements and venous access must also be considered in formulating the PN prescription. Electrolytes are added in accordance with usual requirements; pediatric multivitamin and trace element preparations are generally used as well.

| Nutrient | Start with | Advance by | Goal |
|---|---|---|---|
| Dextrose | 3-4 mg/kg/h | 2-3 mg/kg/h | 8-18 mg/kg/h |
| Amino acids | 1 g/kg/d | 0.5-1.0 g/kg/d | 1.5-3.0 g/kg/d |
| Lipids | 1 g/kg/d | 0.5-1.0 g/kg/d | 1-3 g/kg/d |

**TABLE 42-12. INITIATION OF PARENTERAL NUTRITION IN CHILDREN WITH CANCER**

The risks of PN can be significant, and include infections, hepatotoxicity, suppression of oral intake, and metabolic abnormalities. Most children undergoing aggressive cancer treatment will require indwelling central venous catheters for chemotherapy, but it has been demonstrated that the use of PN increases the risk of infectious complications by 2.4-fold in children receiving chemotherapy who have central access devices in place. [99] The specific role of i.v. fats in increasing infectious complications was evaluated in a randomized trial of more than 500 children and adults undergoing BMT. There was no increased risk of bacteremia or fungal infections in those receiving standard amounts of i.v. lipids (25% to 30% of energy) versus those receiving only 6% to 8% of energy as fat. [100]

Many of the medicines used in oncologic treatment can cause liver injury. The use of PN, alone or in combination with these medicines, may cause biliary dysfunction or steatosis.[101] Another potential risk of PN administration is the generation of peroxide compounds, which may have harmful effects in neonates and others with immune dysfunction or reduced antioxidant defenses.[102] Covering PN bags and i.v. tubing with opaque plastic coloring may reduce this effect. [103]

PN may also cause early satiety and decreased oral intake. In a randomized controlled trial of children and young adults after BMT, outpatient PN delayed resumption of oral intake by 6 days as compared to 5% dextrose hydration. The clinical outcome of both groups was not significantly different. [104] Nausea and vomiting have been linked with children receiving PN at home.[105] Metabolic derangements are also associated with PN. Over- or underhydration, electrolyte imbalance, hyperglycemia, and hypoglycemia are among the most common concerns.[106]

Although the weight of evidence still seems to support a role for PN in selected pediatric cancer patients, close monitoring of laboratory values and clinical condition in addition to the specifics of the PN prescription are necessary to prevent complications ( Table 42-13).

| Parameter | Hospitalized patients on parenteral nutrition | Hospitalized patients on oral/tube feeding | Outpatients on parenteral nutrition | Outpatients on oral/tube feeding |
|---|---|---|---|---|
| Weight | Daily | Daily | Weekly | Monthly |
| Height | Monthly | Monthly | Monthly | Monthly |
| Head circumference (<36 mo) | Weekly | Weekly | Monthly | Monthly |
| Arm anthropometrics | Monthly | As indicated | Monthly | As indicated |
| Intake/output | Daily | Daily | Daily to weekly | Weekly to monthly |
| Electrolytes, glucose | Daily | Weekly | Weekly | Monthly |
| Blood urea nitrogen, creatinine | Weekly | Weekly | Weekly | Monthly |
| Calcium, phosphorus, magnesium | Daily to weekly | Weekly | Weekly | Monthly |
| Triglyceride | Weekly | Monthly | Weekly | As indicated |
| Liver function tests | Weekly | Weekly | Monthly | Monthly |
| Trace elements | Monthly | As indicated | Biannually | As indicated |
| Carnitine | Monthly | As indicated | Biannually | As indicated |
| Vitamin levels | Monthly | As indicated | Biannually | As indicated |

Adapted from Davis AM. Initiation, monitoring, and complications of pediatric parenteral nutrition. In: Walker WD, Walker TD, Davis AM, eds. Pediatric parenteral nutrition. New York: Chapman & Hall, 1997:212.

**TABLE 42-13. NUTRITIONAL MONITORING SCHEDULE OF CHILDREN WITH CANCER**

## GLUTAMINE AND OTHER EXPERIMENTAL NUTRIENTS IN PEDIATRIC CANCER

The significant metabolic and nutritional ramifications of cancer and its therapy have led investigators to evaluate whether the addition of various nutrients may improve clinical outcomes. Some of these trials have used nutrients in high, or pharmacologic, doses; the terms *nutraceuticals* and *nutritional pharmacology* have been coined to describe this use of nutritional therapy. [107]

The amino acid Gln is an example of such a nutrient. Owing to its importance as a fuel source for rapidly turning-over cells, [108] and the demonstration that Gln may reduce amino acid efflux from muscle in catabolic postoperative patients, [109] many studies have been performed to evaluate the clinical efficacy of Gln-supplemented nutrition. Studies have documented a role for Gln in ameliorating the mucosal atrophy seen in prolonged states of PN, [110,111] in the healing of gastrointestinal mucosa after damage from radiotherapy or chemotherapy, [112,113] in improving gut and systemic immune function, [114] and in reducing episodes of bacterial translocation [115,116] and clinical sepsis. [117,118]

Table 42-14 provides an overview of clinical trials of Gln in oncology patients; it is worth noting that the majority of these have been performed in adults. The possibility that the unique metabolic and nutritional requirements of children with cancer may respond differently to Gln supplementation has not been well addressed.

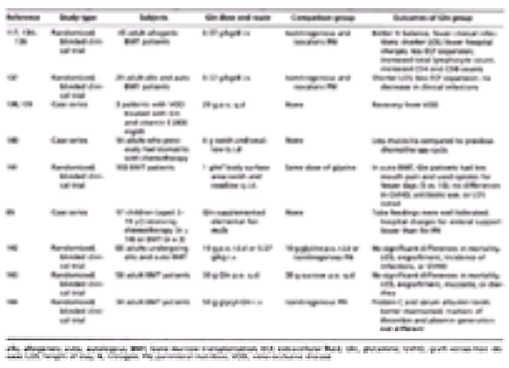

**TABLE 42-14. SUMMARY OF CLINICAL TRIALS OF GLUTAMINE IN THE NUTRITIONAL THERAPY OF PATIENTS WITH CANCER**

## NUTRITIONAL CONCERNS OF LONG-TERM SURVIVORS OF CHILDHOOD CANCER

Most nutritional issues during cancer treatment are associated with weight loss or poor growth, but there is a notable risk of obesity in adults who survive pediatric cancers. As many as half of childhood leukemia survivors in one study became obese young adults. [38] This incidence may be related to the impact of therapy on final adult height. A cohort of BMT survivors, engrafted before or at the onset of puberty, had a significant decrease in final height compared to pretransplant height standard deviation scores. However, most of the patients achieved a height considered to be normal. [119] A study of 33 childhood leukemia survivors followed for a median of 16.2 years found that 36% were obese. All patients had reduced height standard deviation scores during treatment; however, only patients who received cranial radiation with chemotherapy (versus chemotherapy alone) had reductions in final adult height. [120] It has also been postulated that a reduction in physical activity and thus a change in body composition of leukemia survivors may explain the subsequent onset of obesity. [41] A recent report found an alarming incidence of insulin resistance (52%) among 23 long-term survivors of BMT. [121] Unquestionably, complications of oncologic treatment including obesity and insulin resistance need further evaluation and attention. Children should be taught healthy diet principles and acceptable activity options for weight maintenance and control when malnutrition is not a concern (Table 42-9).

## CONCLUSION

The nutritional care of children with cancer is ongoing from the time of diagnosis to many years after treatment. Because children must be expected to continue acceptable growth during extended treatment courses as well as after therapy, serial nutritional assessments should be performed with appropriate actions taken. Malnutrition should not be accepted as an unavoidable consequence of cancer or its therapy. Likewise, every effort should be made in the prevention of obesity in long-term cancer survivors. Extensive nutritional intervention and counseling can assist with the supportive care of the pediatric cancer patient. A multidisciplinary approach considering the quality of life of each child should be used.

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# 43

# SYMPTOM MANAGEMENT IN SUPPORTIVE CARE

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## INTRODUCTION

A diagnosis of cancer evokes immediate fear for patients and their families, in part because cancer is a potentially fatal disease but also because cancer and its treatment are commonly associated with pain, nausea, and other distressing symptoms. Pediatric oncologists have a primary role in symptom management for children with cancer and should reassure patients and their families that relief of distressing symptoms is feasible in most situations.

In this chapter, we summarize aspects of the management of common symptoms, including pain, nausea and vomiting, pruritus, sedation, sleep disturbance, fatigue, dyspnea, dysphoria, constipation or ileus, and urinary retention. In many cases, these symptoms may be interconnected, or treatment of one symptom may exacerbate another. Treatment of symptoms should not proceed in isolation but rather in the context of the patient's overall clinical situation. In some circumstances, treatment of one symptom may cause or exacerbate another symptom, as with sedation or nausea from opioids given to relieve pain. Assessment of the relative contributions of multiple symptoms to the patient's overall quality of life and sense of well-being is essential.

In the United States, the consensus conference on the management of pain in childhood cancer created guidelines for the management of disease- and treatment-related pain and established pain management as a standard of care for these children. [1] The global application of the principles of pain management and palliative care in children with cancer, including the application of the World Health Organization's (WHO) analgesic ladder ( Fig. 43-1), has been a major initiative of the WHO Cancer Unit. A monograph outlining symptom management and palliative care for children is available from the WHO or from the U.S. Cancer Pain Relief Committee.[2]

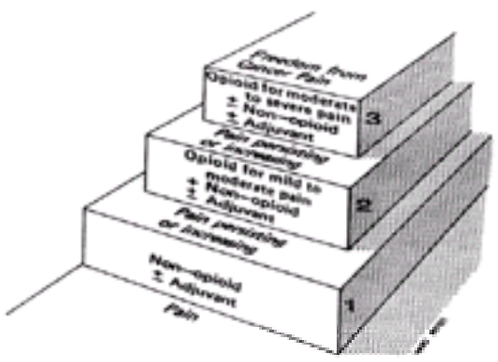


**FIGURE 43-1.** The World Health Organization analgesic ladder. (From Cancer pain relief, 2nd ed. Geneva: World Health Organization, 1996, with permission.)

## PAIN MECHANISMS: IMPLICATIONS FOR TREATMENT

Nociception, or the sensation of tissue injury or inflammation, is an important biologic function that alerts an individual to potential or ongoing injury and prompts the avoidance or limitation of further injury. Lack of protective sensation can lead to a variety of medical complications, including compartment syndromes or decubitus ulcers. Conversely, other types of pain (e.g., metastatic cancer, migraine) often carry no protective significance.

The neural mechanisms underlying nociception have been extensively reviewed, and the reader is referred to other sources for detailed descriptions. [3,4] Nociceptors in deep and superficial tissues can be activated by chemical, thermal, or mechanical stimuli to send afferent impulses through thinly myelinated A-d fibers or unmyelinated C fibers. Primary afferents synapse in the dorsal horn of the spinal cord. Secondary fibers then convey impulses rostrally through a number of tracts, especially the anterolateral spinothalamic tracts. Descending control systems modulate and inhibit pain perception. Endogenous opioids, serotonin, and norepinephrine all appear to be involved in these descending pain-inhibiting systems.

Clinicians and researchers have long observed the lack of correlation between the extent of tissue injury and the intensity of pain or suffering. The experience of pain is subjective and, as such, is modulated by developmental, familial, situational, emotional, and other factors. Assessment of the extent to which one or more of these modulating factors require specific interventions is critical.

*Nociceptive pain* refers to pain associated with intact neurons detecting and transmitting impulses associated with tissue injury or inflammation. The term *neuropathic* pain refers to pain associated with abnormal excitability in peripheral or central neurons. Neuropathic pain may persist even after tissue injury or inflammation have subsided. Neuropathic pain often is described as burning, shooting, or stabbing in character, and it often is associated with paresthesias. The term *allodynia* refers to a condition in which pain can be elicited by normally nonpainful stimuli, such as light stroking of the skin. In the absence of acute inflammation of the skin, allodynia generally implies the existence of an underlying neuropathic condition.

## ASSESSMENT OF PAIN IN CHILDREN WITH CANCER

The experience of pain is known to others only secondhand, and all methods of assessment and measures are imperfect. A distinction commonly is made between *pain assessment*, which involves broad-based clinical hypotheses about the pathophysiology of the pain and its impact on the physical and psychosocial functioning of the patient, and *pain measurement*, which refers to formal scales that assess pain intensity or quality. The management of a child with cancer or treatment-related pain requires ongoing assessment, which in turn requires ongoing measurement of pain to establish the success or failure of treatment strategies.

### Clinical Assessment of Pain

In clinical practice, the measurement of pain contributes to overall assessment of the patient. As the diagnosis and staging of cancer in a child are being established, reports of pain demand a thorough clinical assessment. This assessment is initiated when the practitioner takes seriously the child's report of pain and avoids the common tendency to dismiss or minimize such symptoms that are not easily explained. A medical history is obtained, and the characteristics of the pain are established, including the location, quality, radiation, and relieving and exacerbating factors. Previous opioid use and specific difficulties with opioids should be documented. The history should assess psychological and social factors that may influence the experience of pain. A complete physical examination, followed by appropriate investigations, should seek to establish the cause of the pain. Treatment for the primary cause should be implemented and appropriate analgesia prescribed.

### Measurement of Pain

Pain measures involve one or more of three types of information: self-report (e.g., Visual Analog Scales, [5,6] color analog scales,[7] Faces,[8] Oucher,[9] Poker Chip Tool[10]) behavioral observation,[11] and physiologic measurement. Self-report measures generally are used for children older than 6 years; behavioral scales generally are used in the preverbal and cognitively impaired child. The subsequent discussion is limited to the use of these measurement scales in pediatric cancer pain.

#### *Self-Report Measures*

Patients' reports are the most useful source of information in most circumstances. *Intensity* is the dimension of pain most frequently assessed through self-report measures, although some measures assess qualitative and affective dimensions as well.[12,13] Visual analog scales have been used most commonly for adolescents and adults. These scales usually are presented in the form of a 100-mm line and have two anchors at scale's extremes, the numbers at one end represent no pain, whereas the numbers at the other end represent the worst pain possible. Visual analog scales have useful psychometric properties and are widely used to assess analgesic efficacy. To use these scales, children must be able to interpret the pain using the descriptors and numerals and to understand the concept of proportionality. Visual analog or similar scales have been used successfully by investigators studying acute procedural pain in children with cancer. [14,15]

A number of scales have been developed to extend self-report measurement to younger children, particularly those aged 4 to 8 years. Several scales involve showing the child a series of cartoon faces[8] or photographs[9] that express increasing degrees of distress. Face scales have been used successfully in studies of acute procedural pain in children with cancer.[15] Color analog scales use increasing color intensity to represent increasing pain intensity; they are also useful in the 4- to 8-year-old group.[7,16]

#### *Behavioral Observation*

Behavioral observations are most widely used among children who are cognitively impaired or are too young to use self-report measures. These often include observation of facial expressions, limb movements, crying, and related behaviors. In patients with persistent pain, behavioral observation may underrate pain intensity when compared with self-report.[17] Frequently, children lie immobile in bed, not because they are comfortable but because of severe incidental pain related to movement. Conversely, behavioral scales may overrate pain in the setting of brief procedures and may measure fear or anxiety in addition to pain. Some investigators describe these scales as *distress scales* rather than pain scales, regarding distress as some combination of pain, fear, and anxiety.

The Observational Scale of Behavioral Distress[18] and the Procedure Behavior Checklist (PBCL)[19] are observational scales created to assess procedural pain in children with cancer. Psychometric study has replicated the correlation of the Observational Scale of Behavioral Distress with other measures of pain and distress; however, correlation with physiologic measures may be unstable and in the low range. Validity data of the PBCL indicate that behaviors on the PBCL represent various combinations of pain and anxiety, depending on circumstances and the individual patient, but anxiety tended to be represented more consistently than pain. In addition, behaviors on the PBCL were related more strongly to observer than to patient ratings. The purpose of these measures has been to quantify the occurrence, intensity, and range of a child's pain during traumatic procedures. A novel approach for behavioral observation researchers is to focus on child and adult distress and coping behaviors and, thereby, to discover the processes by which a child's distress may be modified by adult interactions. [15]

Gauvain-Piquard et al.[20] designed a behavioral observation scale to be used in the assessment of tumor-related pain in children between the ages of 2 and 6 years. The 17 items in the scale assessed pain, depression, and anxiety. Some items on this scale lacked operational definitions, which reduced interrater reliability.

#### *Physiologic Measures*

Heart rate, blood pressure, and respiratory rate often increase with increasing pain and decrease in response to analgesics. Although useful, these measures may be confounded by other processes unrelated to pain, including anxiety, hypoxemia, hypovolemia, and fever. Autonomic measures may be the only signs of pain in the intensive care patient receiving neuromuscular blockade; absence of movement does not ensure unconsciousness or comfort. Physiologic signs may habituate in the setting of chronic pain.

## PATHOPHYSIOLOGY OF TUMOR-RELATED PAIN IN CHILDHOOD CANCER

Tumor commonly causes pain from involvement of bone, viscera, and nerves. Visceral stretch, compression, or ischemia activates nociceptive endings that evoke pain. Visceral pain typically is more poorly localized than is somatic pain. Animal studies have demonstrated primary visceral afferents that discharge maximally only to apparent noxious stimuli and have small myelinated or unmyelinated axons.

Mechanisms that may cause pain from bone metastases include stimulation of nerve endings in the endosteum by chemical agents released from the destroyed bone tissue, such as prostaglandins, bradykinin, substance P, or histamine; stretching of the periosteum; fractures; and tumor growth into surrounding nerves and tissues. [21] The periosteum is more sensitive than bone marrow and cortex.

Mechanisms for persistent neuropathic pain after damage to peripheral tissues have been reviewed.[22,23] They include deafferentation-induced hyperactivity of dorsal horn pain transmission cells, loss of central inhibition by loss of myelinated primary afferents, ectopic impulse generation in damaged nociceptive primary afferents, sympathetic efferent activation of damaged or intact primary afferents, and changes in autonomic reflexes. Systematic evaluation and consideration of the neurologic mechanisms that underlie patterns of referral and pain quality should be encouraged. [24]

## EPIDEMIOLOGY OF CANCER PAIN IN CHILDHOOD

Since Farber et al.[25] described the use of folic acid antagonists in the treatment of childhood leukemia, survival from childhood cancer has dramatically improved.

Improvement in survival has resulted from the evolution of multimodal treatment regimens. Since the development of multimodal treatment, the epidemiology of pain associated with childhood cancer has changed from a predominantly tumor-related to a predominantly treatment-related pattern.

At diagnosis and during the early treatment phase of childhood cancer, tumor-related pain predominates. Miser's study [26] of children with non–central nervous system malignancy at the National Cancer Institute found that 62% of the children studied presented to their practitioner with a report of pain before the diagnosis of cancer and that the pain had persisted for a median of 74 days before treatment was begun. The duration of pain experienced by patients with metastatic disease was not longer than that for patients without metastases. After initiation of cancer therapy, most children had resolution of pain related to their tumors; only the rare patient experienced persistent pain. Pain persisted for a median of 10 days after the institution of cancer therapy. Children with hematologic malignancies had a shorter duration of pain after initiation of treatment than did children with solid tumors.

We have accrued little information about the epidemiology of pain related to pediatric brain or spinal cord tumors. Children with brain tumors present to their practitioners with symptoms consistent with raised intracranial pressure, including headache, or with abnormal neurologic signs (see Chapter 27). A retrospective review of children with spinal cord tumors at the Children's Memorial Hospital in Chicago showed that most children with spinal cord tumors do present to their practitioners with pain.[27] Metastatic spinal cord compression is unusual at diagnosis and is more likely to occur later in a child's illness. Back pain, more often than abnormal neurologic signs or symptoms, is the initial presenting sign of spinal cord compression in children. [28]

As the treatment evolves, treatment-related rather than tumor-related causes of pain predominate. [29,30] Possible causes of treatment-related pain include mucositis, phantom limb pain, infection, antineoplastic therapy–related pain, postoperative pain, and procedure-related pain (e.g., needle puncture, bone marrow aspiration, lumbar puncture, and removal of central venous line).

Tumor-related pain frequently recurs in patients at the time of relapse and during the terminal phase of an illness. Palliative chemotherapy and radiotherapy, depending on tumor type and sensitivity, sometimes are instituted as modalities of pain control for terminally ill pediatric patients with malignancy. Severe pain in terminal pediatric malignancy occurs more commonly in patients with solid tumors metastatic to spinal nerve roots, nerve plexus, or larger peripheral nerves, or with spinal cord compression.[31]

## SYNDROMES OF PEDIATRIC CANCER PAIN

### Treatment-Related Syndromes

#### *Mucositis*

The optimal management of pain related to cancer chemotherapy and radiation-induced mucositis is not established. A national survey of U.S. hospitals indicated a wide variation in the prophylaxis and management of oral mucositis in the adult population and lack of a consistent approach to pain management despite National Institutes of Health guidelines. [32] Therapy for mucositis has included topical therapies, either singly or in combination, including saline, sodium bicarbonate, hydrogen peroxide, nystatin, viscous lidocaine, dyclonine, as well as systemic therapies, especially opioids and systemic antifungal agents. Hospitals vary greatly in their choice of treatments for mucositis. Hospitals frequently have second- and third-line regimens for the management of mucositis, including opioids, but no consistent pattern can be identified.

The mucositis that occurs after conditioning for bone marrow transplantation is more intense and prolonged than that associated with routine chemotherapy. The pain of mucositis in transplantation patients typically is continuous, with exacerbation during mouth care, swallowing, and on awakening. Even when treated with opioids, the patient may have pain that can preclude talking, eating, and swallowing. [33] For patients with severe mucositis, parenteral opioids are appropriate, administered as either a continuous infusion or patient-controlled analgesia (PCA). Data are emerging regarding the safety and efficacy of PCA in the setting of mucositis pain after bone marrow transplantation in children.[33,34,35] and [36] In some studies, PCA produced better analgesia than did continuous infusions or nurse-administered intermittent dosing,[35] whereas in other studies, analgesia was equivalent [37] but, almost invariably, patients receiving PCA achieved either equivalent or better analgesia despite using less opioid than patients who received the drug by infusion or nurse-administered intermittent dosing. A randomized comparison of PCA morphine, hydromorphone, and sufentanil found similar analgesia in most respects, although a higher percentage of patients in the sufentanil group failed to achieve adequate analgesia, whereas morphine was the preferred choice in terms of fewest side effects.[38] Sufentanil developed tolerance to twice the degree of morphine or hydromorphone.

Some centers use topical agents, including local anesthetics, for analgesia in patients with mucositis. The upper limit for repeated dosing for oral mucosal lidocaine is not established; a single dose of lidocaine 2% (5 mL, swished, not swallowed) resulted in safe plasma concentrations. [39] Some practitioners still have concerns that topical anesthesia may extend to supraglottic and glottic structures, thereby creating a predisposition to aspiration of secretions. The incidence of this complication after oral mucosal lidocaine use is unknown.

#### *Graft-Versus-Host Disease*

The gastrointestinal manifestation of acute graft-versus-host disease (GVHD) may be associated with severe abdominal pain. After mucositis, GVHD is the next most common cause of pain subsequent to allogeneic bone marrow transplantation. Pain due to GVHD frequently requires the administration of opioids. [34]

#### *Phantom Limb Pain*

Phantom sensations and phantom limb pain are common among children after amputation of an extremity for cancer. The incidence and severity of phantom pain in children tend to decrease with time after amputation.[40,41] Evidence appears to demonstrate that preoperative pain in the diseased extremity may be a predictor for subsequent phantom pain.[40] One study suggested that prior treatment with chemotherapy increased the risk of phantom pain after subsequent amputation. [42] In some studies in adults, preoperative regional anesthesia appeared effective in preventing phantom pain, [43,44] whereas in other studies, no preventive effect could be demonstrated.[45] Differences in results may relate to the study design and to the method of regional anesthesia, drug choice, and the duration of interruption of afferent inputs to the spinal cord. Tricyclic antidepressants and calcitonin [46] have undergone positive clinical trials for phantom limb pain.

Although traditionally opioids were regarded as ineffective for phantom pain, a recent study found useful analgesic effects in a subset of patients. A preliminary report appears to confirm the common impression that early and frequent use of a limb prosthesis may reduce the duration and severity of phantom pain. [47]

#### *Infection*

Pain is commonly associated with infection in the child with cancer and usually is associated with an acute illness. Common causes of pain due to infection in the immunocompromised child include perioral, perirectal, and skin infection (particularly at sites of intravenous access). Resolution of pain usually is associated with resolution of the infection, and primary therapy should be directed at treatment of the infection.

Acute herpes zoster commonly is associated with pain. Unlike in adults, in children, herpes zoster infection is rarely associated with post-herpetic neuralgia. A small subgroup of children and adolescents, however, develop severe burning pain and skin hypersensitivity that persists years after herpetic infections. Growing evidence from adult studies points to a reduction in the incidence of post-herpetic neuralgia after early treatment with antiviral agents. [48,49] In adults, treatment for post-herpetic neuralgia includes tricyclic antidepressants [50,51]; anticonvulsants (including gabapentin) [52]; topical, regional, and systemic local anesthetics; opioids; and epidural injection of local anesthetics and corticosteroids. A recent study reported benefit from spinal administration of corticosteroids. [53]

#### *Antineoplastic Therapy–Related Pain*

Peripheral venous injection of chemotherapy (e.g., leucovorin, thiotepa) may be associated with local pain at the time of injection. After injection of chemotherapy into a peripheral vein, thrombophlebitis occasionally develops. Intrathecal chemotherapy has been associated with arachnoiditis and meningeal irritation syndrome (i.e.,

headache, nuchal rigidity, fever, nausea, and vomiting).

Extravasation of antineoplastic agents is becoming less problematic in the treatment of cancer in children because of the increased use of central venous lines and intravenous access devices. Children who do not have these devices are at risk for extravasation. This is a particular danger for antineoplastic agents that have vesicant or irritant properties. Vesicants, when extravasated, produce local necrosis, and irritants produce burning or inconsequential inflammation without necrosis. A long-term pain management problem may be associated with painful vincristine neuropathy. Although in most children with cancer this usually is a self-limiting condition, it may, in some cases, cause severe pain that requires treatment with strong opioids, anticonvulsants, or antidepressants.

As new antineoplastic agents become available for the treatment of childhood cancer, the practitioner must be aware of the possibility of new pain syndromes arising. The monoclonal antibody 3F8 is an antiganglioside agent being explored as a novel agent for the treatment of stage IV neuroblastoma. The infusion of this agent has been associated with severe abdominal and limb pain in children, despite opioid administration. Infusion of this antibody into rats produces an acute painful peripheral neuropathy. In the rodent model, both gabapentin and intravenous lidocaine appear effective. [54]

### *Postoperative Pain*

The management of postoperative pain in the child with cancer is similar, in many respects, to the management of postoperative pain in a patient without malignancy, as has been reviewed elsewhere. Nonetheless, some features in postoperative care are unique to the child with cancer. In a patient who was receiving opioids preoperatively, the daily dose of opioids should be calculated, and this dose should be used as a baseline to which additional opioids are added for the purposes of postoperative pain control. Because this principle is commonly ignored in postoperative care, oncology patients often are dramatically undermedicated. Opioid requirements can be very high in children undergoing extensive thoracic, abdominal, pelvic, or extremity cancer resections.

We have been informed of several incidents in which pediatric oncology patients were distressingly aware intraoperatively during anesthesia due to selection of a nitrous oxide–opioid–relaxant-based anesthetic technique that did not sufficiently take into account the patients' increased opioid requirements (Charles B. Berde, *unpublished observations*). Unless contraindicated by severe hemodynamic instability, oncology patients should receive sufficient depth of anesthesia with inclusion of volatile or intravenous anesthetic agents to diminish as much as possible the likelihood of intraoperative awareness. Even among children with severe doxorubicin (Adriamycin) cardiotoxicity, sufficient doses of sedative-hypnotic agents can be incorporated into the anesthetic plan.

### *Procedure–Related Pain*

#### Needle Puncture

Needle puncture often is required in cancer treatment for obtaining blood specimens, administering intravenous or intramuscular chemotherapy (e.g., asparaginase), or obtaining access to implanted intravenous access devices. Needle procedures are a major source of distress for children. Children must be adequately prepared before their first needle puncture to minimize their fear and anxiety. This preparation commences with the practitioner working with a child's parents to obtain an insight into the child's coping style, to explain to them the nature of the procedure, and to enlist their support. An age-appropriate explanation to the child should follow, with consideration of a particular child's previous experience and coping style. [55]

A variety of topical treatments have been used to provide analgesia for needle procedures. Skin cooling with ice or fluorocarbon coolant sprays has been used with some success. The eutectic mixture of local anesthetics (EMLA) has become a useful method of topical anesthesia before venipuncture, venous cannulation, lumbar puncture, and accessing of subcutaneous central venous ports. [56,57] EMLA has proven safe, with low plasma concentrations of local anesthetic and a negligible risk of methemoglobinemia. Although it provides no analgesia for structures deep to the dermis, EMLA can help to lessen the pain of subsequent deeper infiltration with local anesthetic. Skin cooling and EMLA can produce vasoconstriction, [58] which may occasionally make venous cannulation more difficult. More commonly, though, these procedures reduce distress sufficiently to facilitate cannulation, thereby outweighing the potential disadvantage of vasoconstriction. The depth of penetration of this anesthesia increases in proportion to the duration of the application of EMLA. [59] Although 60 minutes is the commonly recommended application span before a procedure is undertaken, a longer application period is recommended for more distressing procedures. In addition, application for 3 hours or longer tends to produce vasodilation, [58] rather than vasoconstriction, probably due to a combination of direct actions on subdermal vascular smooth muscle tone and conduction blockade at sympathetic terminals in subdermal arterioles. To achieve the benefits of 3-hour application in outpatients, application of EMLA at home before coming to clinic is advised.

Several other topical local anesthetics also appear to be effective. A formulation of tetracaine (amethocaine; Ametop) is available in many countries (although not in the United States). This tetracaine formulation provides faster onset of cutaneous analgesia than does EMLA [60,61] and showed better efficacy in some studies. [61,62] Tetracaine has the advantage of inciting vasodilation at all times after application, which may facilitate identification of veins for venipuncture or venous cannulation. Liposomal lidocaine formulations have become available in the United States, and these too appear to provide rapid onset of cutaneous analgesia. [63]

EMLA and other forms of topical anesthesia are very useful, and their application should be encouraged, but they should not be promised as a panacea to relieve all needle-related pain. If venipuncture is unsuccessful at sites treated with EMLA, it may be necessary to perform subsequent attempts at untreated sites.

#### Lumbar Puncture

The pain related to lumbar puncture is caused largely by puncture of the skin by the spinal needle or by contact with bone if the needle fails to pass directly through the interspace. Although the dura is a pain-sensitive structure, patients only rarely experience pain as the dura is punctured or as intrathecal medication is instilled. EMLA or other topical anesthetics may be applied before the subcutaneous administration of local anesthesia. Equally important factors in the distress of lumbar puncture for children are the required body position and the necessity to remain still until the procedure is complete. For children who are unable to comply voluntarily, a variety of cognitive and behavioral techniques, conscious sedation, or even general anesthesia may prove beneficial. Hip flexion facilitates needle placement. Conversely, despite common practice, neck flexion is irrelevant to the degree of flexion or extension of the lumbar spine, and is not necessary. In infants, neck flexion may predispose to airway obstruction, particularly if sedatives are administered.

Occasionally, lumbar puncture may produce a sustained cerebrospinal fluid leak, leading to low intracranial pressure and so-called spinal headache. The epidemiologic pattern of postdural puncture headache in children with cancer has been examined in recent studies. [64] Dural puncture headache is not rare among children receiving diagnostic lumbar puncture, with an incidence of 11%, 9%, and 8% in three studies. [64,65] and [66] Headaches occurred at a similar frequency among toddlers, school-age children, and adolescents. [64]

The treatment of this headache, if it occurs, is accomplished with simple analgesics, adequate hydration, and a recumbent position. In refractory cases, an epidural blood patch (i.e., the injection of autologous blood in the epidural space) may be required to alleviate this symptom. [67,68] For a child with a history of severe postdural puncture headache who requires subsequent lumbar punctures, consideration should be given to use thin (24- to 27-gauge) spinal needles with modified noncutting tips. An extensive body of literature addressing the subject of adults receiving spinal anesthesia indicates that these needles substantially reduce the likelihood of spinal headache in surgical and obstetric patients. [69] Use of these needles entails a learning curve. Withdrawal of cerebrospinal fluid is much slower with narrow needles and may require gentle syringe aspiration. Measurement of cerebrospinal fluid pressure probably is less reliable through these narrow needles.

#### Bone Marrow Aspiration

The pain related to bone marrow aspiration results from the insertion of a large needle through the periosteum of the posterior superior iliac spine or other sites and from the unpleasant sensation experienced at the time of suctioning of the marrow. The latter pain is not alleviated by the administration of local anesthetic. The almost universal distress of children undergoing bone marrow aspiration was documented previously. [70,71] Conscious sedation, [72] general anesthesia, and a variety of cognitive-behavioral interventions (including guided imagery, relaxation, and hypnosis) [73] have shown some effectiveness in alleviating pain and distress in this setting. [74] No single method can be advocated over another, because the choice depends on the patient's age, preference, previous experience with the procedure, and temperament and on the availability of services. For example, a 12-year-old child who is an excellent hypnotic subject and who experiences severe nausea or dysphoria with sedation or general anesthesia may prefer hypnosis to pharmacologic measures. Conversely, a 3-year-old child who has had traumatic experiences

with previous procedures may fare better with a brief general anesthetic.

Propofol, either alone or in combination with opioids, has emerged as a useful short-acting intravenous general anesthetic for pediatric oncologic procedures, [75] particularly for children with indwelling central venous access. Alternatives for children with difficult or distressing intravenous access include inhalation general anesthesia, sedation using 50% nitrous oxide by inhalation, [76] oral administration of midazolam and ketamine,[77] or oral transmucosal fentanyl citrate (OTFC).[78]

In a study of patients undergoing bilateral iliac bone marrow harvest, unilateral bupivacaine infiltration around the periosteum reduced pain scores for 3 days relative to the ratings for the contralateral side.[79] When bupivacaine is injected in this setting (maximum dose 2.5 mg per kg), the practitioner must take extra care to avoid the uncommon but serious occurrence of inadvertent injection directly into the marrow through a previous needle hole in the cortex. Systemic uptake of local anesthetic from the marrow is very rapid (analogous to intraosseous drug administration in resuscitation), leading to the potential for severe cardiac depression and convulsions.

### Removal of Central Venous Lines

The increasing use of tunneled central venous lines in children with cancer has created the need for sedation or for brief general anesthesia during the lines' removal. Common practice at our institution calls for anesthesiologists to administer propofol through the line prior to the procedure and then to maintain anesthesia with inhalation agents as needed.

### Other Sources

The spectrum of colony-stimulating factor–related toxicities in children is similar to that reported in adults, but the effects may occur less frequently. The predominant side-effect associated with administration of filgrastim (G-CSF) or sargramostim (GM-CSF) is medullary bone pain, and this effect is dose-related. The pain associated with myeloid growth factors usually responds to non-opioid analgesics, although opioids may be required for some patients.

### Tumor-Related Syndromes

Aside from the predominance of treatment-related pain, a number of children experience pain related to tumor, despite the initial response of their pain to treatment. Miser[79] found that one-third of the pain experienced by patients in the hospital setting was tumor-related pain, but less than 20% of the pain experienced by outpatients was caused by tumor.

Direct tumor involvement of bone, hollow viscera, or nerves is a common cause of pain in adult patients with cancer. Such tumor involvement commonly results in somatic, visceral, and neuropathic pain, respectively. Somatic pain is typically well localized and is frequently described as aching or gnawing. Examples of somatic pain include pain associated with primary or metastatic bone disease or postsurgical incisional pain. Visceral pain results from the infiltration, compression, distention, or stretching of thoracic and abdominal viscera by primary or metastatic tumor. This pain is poorly localized but often is described as deep, squeezing pressure and may be associated with nausea, vomiting, and diaphoresis, particularly when acute. An example of visceral pain is pain associated with liver tumor, either primary (e.g., hepatoblastoma) or metastatic (e.g., neuroblastoma). Neuropathic pain most commonly results from tumor compression or infiltration of peripheral nerves or the spinal cord. Chemical- or radiation-induced injury also may result in this sort of pain. The clinical features of pain resulting from neural injury include abnormal or unfamiliar unpleasant sensations (dysesthesias), pain evoked stimuli that are normally innocuous, such as light touch of the skin (allodynia), pain felt in a region of a sensory deficit (anesthesia dolorosa), and pain characterized by burning or electrical quality or paroxysmal brief shooting component.

### Pain Syndromes Unrelated to Treatment or Tumor

The pediatric oncology patient is just as prone to pain unrelated to the tumor or its treatment (e.g., trauma, migraine, nonspecific abdominal pain) as is any child in the general pediatric population. Diagnosis generally occurs after causes related to the tumor or its treatment are excluded. Acute appendicitis in children with leukemia and other malignancies may present a diagnostic dilemma and is associated with a high diagnostic error rate. [80]

### Chronic Pain as a Management Problem for the Long-Term Survivor of Cancer

As the survival for children with cancer improves, practitioners must be alert to the potential for chronic pain management problems to arise as a consequence of treatment. There is considerable literature about the potential physical and psychological late effects of childhood cancer treatment but little about chronic pain as a consequence of treatment.

At the Pain Treatment Service of the Children's Hospital of Boston, patients who are long-term survivors of childhood cancer and who have chronic pain management problems often are seen. A variety of problems have been encountered in these patients, including chronic abdominal pain of uncertain cause, causalgia of the lower extremity, phantom limb pain, chronic lower extremity pain due to a mechanical problem with an internal prosthesis, avascular necrosis of multiple joints, neuralgia and mechanical pain due to failure of bony union after tumor resection, and post-herpetic neuralgia. A number of these patients require chronic opioid therapy as part of their pain management strategy.

## PSYCHOLOGICAL INTERVENTIONS AND OTHER NONPHARMACOLOGIC APPROACHES TO PAIN MANAGEMENT

Preparation of the child, including a description of the steps of a given procedure and of the sensations experienced, is perhaps the most common intervention for children about to undergo invasive medical procedures. The rationale for this intervention is that coping with unexpected stress is difficult and more anxiety-provoking than is coping with anticipated or predictable stress. [79] It is helpful to perform procedures in a quiet, calm environment conducive to reducing stress and anxiety, in a location separate from the child's room.

Nonpharmacologic methods of pain control in children include a variety of techniques that are categorized as physical (e.g., massage, heat and cold stimulation, electrical nerve stimulation, acupuncture), behavioral (e.g., exercise, operant conditioning, relaxation, biofeedback, modeling, desensitization, art and play therapy), or cognitive (e.g., distraction, attention, imagery, thought stopping, hypnosis, music therapy, psychotherapy), according to whether the intervention is focused on modifying an individual's sensory perception, behaviors, or thoughts and coping abilities.

Complementary and alternative treatments are widely used by children with cancer, often without the knowledge of their physicians. Physicians should include questions about use of these treatments in a patient's medical history. Because these techniques are so widely used, a need exists for properly controlled studies to define safety and efficacy.[82] Placebo effects are highly prevalent, and study design issues can be complex.

Cognitive-behavioral techniques are most commonly used for the pediatric cancer patient to decrease distress and enhance a child's ability to cope with medical procedures. The decision to use a psychological or pharmacologic approach (or both) depends on the practitioner's knowledge of and skill with the procedure, an understanding of the child, and the expectations of pain and anxiety for that particular child undergoing a specific procedure. Choosing which nonpharmacologic method to use is based on factors such as the child's age, behavioral factors, coping ability, fear and anxiety, and the type of pain experienced.

The effectiveness of hypnosis in the reduction of pain and anxiety during bone marrow aspiration and lumbar puncture in children has been confirmed by several reports.[73,83] Similarly, the role of distraction techniques in reducing children's distress during procedures has been examined by several investigators and has been shown to be effective. Distraction was less effective for younger children in one study. [84] Another study enlisted the support of parents and showed a reduction in the children's behavioral distress and a lowering of the parents' anxiety. [15] Several investigators have examined and demonstrated the effectiveness of cognitive-behavioral interventions comprising multiple components, including preparatory information, relaxation, imagery, positive coping statements, modeling, and behavioral rehearsal.[85,86]

Cognitive-behavioral approaches have a distinct advantage in that learning them for one situation may be generalized to their use in another situation. For example, a child who learns relaxation training and guided imagery to manage needle procedures then may apply this method to managing headache, dyspnea, nausea, or other symptoms.

## ANALGESIC MEDICATIONS

### Nonopioids: Acetaminophen, Aspirin, and Nonsteroidal Antiinflammatory Drugs

Acetaminophen is the nonopioid analgesic used most commonly in children. It inhibits prostaglandin synthesis primarily in the central nervous system and lacks the sedative effects that characterize opioids. Acetaminophen provides, at most, a minimal antiinflammatory effect, unlike nonsteroidal antiinflammatory drugs (NSAIDs), which inhibit peripheral cyclooxygenases. Acetaminophen lacks the peripheral side effects of gastritis and inhibition of platelet function found with aspirin and NSAIDs. There is a potential for hepatic and renal injury, but this is extremely uncommon with therapeutic doses. [87] Acetaminophen has antipyretic action and may be contraindicated in circumstances in which it is important to monitor a fever. Pediatric dosing of acetaminophen is based on the dose-response relation for antipyretic effects, because proper dose-response studies for analgesia are limited. Oral dosing of 15 mg per kg every 4 hours appears to be safe. Although rectal dosing generally is avoided in oncology patients, recent studies suggest that rectal absorption is inefficient and that single rectal doses of 30 to 40 mg per kg can be given without generating excessive plasma concentrations.[88]

Aspirin and NSAIDs often are contraindicated in pediatric oncology patients, who commonly are at risk for bleeding due to thrombocytopenia. Aspirin's effects are a greater concern because aspirin's irreversible inhibition of platelet function persists for many days after the drug is cleared, in contrast to the reversible inhibition seen with NSAIDs, which terminates as the drug is cleared. In a comparative study of aspirin and ibuprofen in children with juvenile rheumatoid arthritis, the drugs were equally efficacious, but the dropout rate because of side effects was significantly higher in the aspirin group. [89] In selected children with adequate platelet number and function, NSAIDs may be extremely helpful analgesics, alone and in combination with opioids. It is a common misperception that NSAIDs are specifically effective for bone pain, but a meta-analysis of NSAID use for pain relief in adult cancer patients found no basis for a specific effect on bone versus visceral or other pains. [90]

Choline magnesium salicylate (Trilisate) and related nonacetylated salicylates are widely recommended because of reports of minimal effects on platelet function in adults.[91] However, clinicians should view these data with some caution, because the studies do not include patients with severe thrombocytopenia. The safety of choline magnesium salicylate is not established for patients with active bleeding or a platelet count of fewer than 20,000 cells per cubic millimeter.

A new class of NSAIDs, the cyclooxygenase-2 (COX-2) inhibitors, have been developed to target a specific isoenzyme (found predominantly in leukocytes, peripheral nerves, and the central nervous system) that is involved in generation of prostanoids, which contribute to pain and inflammation. [92] COX-2 inhibitors exert minimal inhibition of COX-1, which makes them less prone to cause gastritis [93,94] or platelet dysfunction.[95] The two COX-2 inhibitors currently available in the United States, celecoxib and rofecoxib, have not undergone pediatric clinical trials. We make use of these drugs off-label for children with specific indications. It is likely that after further study, these agents will assume an important role in pain management for children with cancer. One study examined bleeding time; *in vitro* platelet aggregation to collagen, arachidonate, or a thromboxane receptor agonist; and serum thromboxane $B_2$ concentrations in normal volunteers randomized to receive celecoxib, placebo, or a traditional NSAID, naproxen.[95] For all the preceding end points, naproxen increased *in vivo* and *in vitro* measures of bleeding diathesis as compared with placebo, whereas celecoxib showed no differences as compared with the placebo group. We were unable to identify studies of *in vitro* or *in vivo* hemostasis among patients with moderate or severe degrees of thrombocytopenia receiving COX-2 inhibitors.

### Weak Opioids: Codeine

The distinction between weak opioids and strong opioids in the WHO analgesic ladder is a matter of dose as well as choice of agent. In pediatrics, codeine is the most commonly used agent among the weak opioid class. Although codeine sometimes is accused of having a “ceiling effect,” evidence for this is lacking. The practical limitation on escalated dosing of codeine is that side effects increase with dosing greater than 2 mg per kg. Codeine typically is administered to pediatric patients in oral doses of 0.5 to 1.0 mg per kg every 4 hours. Codeine is largely a prodrug; it is O-demethylated to morphine as the active agent. Recent pharmacogenetic studies have demonstrated that 4% to 14% of subjects lack the hepatic enzyme functions for conversion of codeine to morphine; in these subjects, codeine is largely ineffective as an analgesic. [96,97] Thus, if a patient obtains little analgesia with codeine, clinicians should not hesitate to substitute a different opioid.

Oxycodone may also be used as a weak opioid in lower doses (e.g., 5 mg for adolescents), but dose escalation is highly effective, and no evidence of a ceiling effect exists.

So-called weak opioids are practical in a situation in which opioids are needed immediately by a patient who is far from the prescribing physician and requires a prescription issued by telephone to the pharmacy. In some locations in the United States, telephone prescribing of combinations of acetaminophen with codeine (e.g., Tylenol 3 or 4) or hydrocodone (e.g., Vicodin) is permitted, but telephone prescription of strong opioids is prohibited. Some pharmacies will allow opioid prescriptions to be faxed for patients who are in palliative care.

In some countries, legal barriers to the prescribing of mixed agonist-antagonist opioids or opioids with preferential action at k receptors are less restrictive than those for standard μ-opioid agonists. The k-agonist buprenorphine may have a useful role for children in countries having limited availability of morphine. [96] Aside from availability issues, indications for use of mixed agonist-antagonist opioids or opioids with preferential action at k receptors in children are few at present. Mixed agonist-antagonists frequently produce somnolence or dysphoric reactions in adults, and they may evoke withdrawal symptoms in patients who have been receiving μ-opioid agonists.

### Strong Opioids: Morphine, Hydromorphone, Fentanyl, Meperidine, and Methadone

For moderate to severe pain, μ-opioid agonists are indicated. The starting dosage schedule for the opioids commonly used in pediatric patients is shown in Table 43-1. Morphine is the most widely used strong opiate and is a proper first choice in most circumstances. It may be administered by the oral, rectal, intravenous, subcutaneous, epidural, intrathecal, or intraventricular route. Morphine has significant first-pass metabolism in the liver after oral dosing, and an oral-to-parenteral ratio of approximately 3:1 commonly is recommended. The major metabolite of morphine, morphine-6-glucuronide, produces considerable analgesia and side effects comparable to morphine with chronic dosing.[98,99] A typical starting dose for immediate-release oral morphine in opioid-naive subjects is 0.3 mg per kg every 4 hours. Morphine-6-glucuronide may accumulate and exacerbate sedation in patients with renal insufficiency, because it requires renal clearance. Because morphine clearance is delayed in the first 1 to 3 months of life, [100] starting doses in very young infants should be reduced by approximately 50% on a per-kg basis relative to dosing recommended for older children.

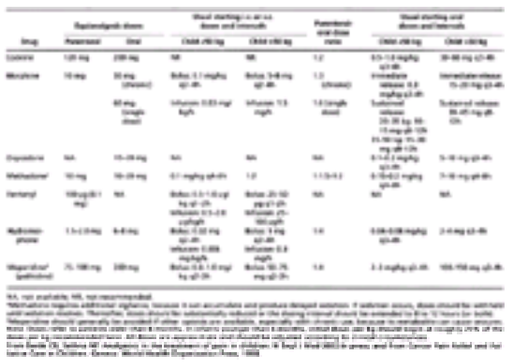

**TABLE 43-1. OPIOID ANALGESIC INITIAL DOSAGE GUIDELINES**

Sustained-release preparations of morphine and oxycodone are available. They permit oral dosing at intervals of twice or three times daily. In a study of children with cancer, sustained-release morphine given twice daily produced greater fluctuations in plasma concentrations than was anticipated, supporting the recommendation that the drug be administered three times daily.[101] Crushing slow-release tablets produces immediate release of the drug, which limits their use for children who are

unable to swallow pills. Sustained-release oral suspensions of morphine are under investigation.

Hydromorphone is an opioid available for oral, intravenous, subcutaneous, epidural, and intrathecal dosing. Hydromorphone commonly is used in adults if there are dose-limiting side effects from morphine. Adult studies indicate that hydromorphone is five to eight times as potent as morphine. A double-blind, randomized, cross-over comparison of morphine to hydromorphone using PCA in children and adolescents with mucositis after bone marrow transplantation showed that hydromorphone was well tolerated and had an approximate potency ratio of 5:1 relative to morphine in this setting. [36] Hydromorphone is convenient for subcutaneous infusion if a high-potency, high-concentration agent is desired to limit infusion volumes, because it is commercially available in 10-mg-per-milliliter solutions and can be prepared in concentrations up to 50 mg per milliliter if needed. The metabolites of hydromorphone are under investigation.

Fentanyl is a synthetic opioid that is approximately 50 to 100 times more potent than morphine, depending on whether infusion or intravenous single-dose comparisons are used. Fentanyl has a very rapid onset after intravenous administration because of its high lipid solubility and rapid entry into the brain. Its duration of action after intravenous bolus administration is much shorter than that for morphine. These features make fentanyl especially useful for brief noxious procedures, for which rapid onset and short duration are useful. Fentanyl may also be used for continuous infusion for selected patients with dose-limiting side effects from morphine, especially pruritus. Although the central mechanisms of pruritus can be activated by fentanyl, less peripheral excitation of itching may ensue with this drug because of less histamine release from fentanyl than with morphine.

The high lipid solubility of fentanyl permits two novel routes of administration: transdermal and oral transmucosal (OTFC). Transdermal administration using a patch permits sustained analgesic effect for selected patients who are unable to tolerate oral opioids. [102] It should not be used in opioid-naive patients and should not be used in patients with rapidly changing pain intensity. OTFC produces a rapid onset of effect and bypasses first-pass hepatic clearance. Schechter et al. [78] described the successful use of oral transmucosal fentanyl for sedation or analgesia for bone marrow biopsy or aspiration and lumbar puncture. Vomiting appears less frequent among pediatric oncology patients than among patients receiving OTFC for sedation prior to surgery.

Meperidine is a synthetic opioid that has been used for procedures and postoperative pain, although it has no advantages over morphine for the latter. A major drawback to the prolonged use of meperidine is that its major metabolite, normeperidine, can cause dysphoria, excitation, and convulsions, particularly in patients with impaired renal clearance. Meperidine is an acceptable alternative to fentanyl for short painful procedures; its duration of action is shorter than that of morphine. Meperidine in low doses (0.25 to 0.5 mg per kg intravenously) is uniquely effective among the opioids for treatment of rigors after the infusion of amphotericin or blood components.

Methadone is a synthetic opioid that has a prolonged duration of action owing to slow hepatic metabolism. In a single parenteral dose in opioid-naive subjects, its potency is similar to that of morphine. It is absorbed efficiently after oral administration, with an oral to parenteral ratio of approximately 1.5 to 2:1. Methadone clearance is variable, with elimination half-lives ranging from 6 to 36 hours. In children undergoing surgery, methadone produced more prolonged analgesia than morphine.[103]

In the past, titration of methadone has been regarded as difficult, and it has been commonly observed that patients converted from a regimen of other opioids to methadone developed delayed somnolence. This outcome often was ascribed to the variability in methadone pharmacokinetics. Although kinetic factors play some role, recent studies have identified additional biologic bases for difficulties in conversion of other opioids to methadone.

Methadone is supplied as a racemic mixture. The L-isomer is a standard μ-opioid, whereas the D-isomer (and, to a lesser extent, the L-isomer) acts as an antagonist at the NMDA subgroup of excitatory amino acid receptors.[104,105] NMDA receptor antagonists have been shown to prevent and partially reverse the development of tolerance to μ-opioids.[105] The implications of this are twofold: First, the potency ratio of methadone to morphine and other opioids depends on the preexisting degree of opioid tolerance. If methadone is given to opioid-tolerant subjects, it acts much more potently than it would in opioid-naive subjects. Second, dosing of opioid-tolerant subjects according to tables derived for opioid-naive subjects will produce overdoses. Ripamonti et al. [106] have examined conversion ratios from morphine to methadone in adults and derived a regression formula based on previous daily oral morphine dosing. Our opinion is that the conversion ratios derived from adult studies by Ripamonti et al. should be used as an approximate starting point for children in lieu of more specific pediatric data.

Because of these factors, frequent patient assessment is key to safe and effective use of methadone. If the patient becomes comfortable after initial doses, the dose should be reduced or the interval extended to reduce the likelihood of subsequent overdosage. If a patient becomes oversedated early in dose escalation, the recommendation is to stop dosing (rather than simply to reduce the dose) and to observe the patient until he or she exhibits increased alertness. Although as-needed dosing is discouraged for most patients with cancer pain, some clinicians find it a useful way to establish a dosing schedule for methadone. Methadone is especially convenient as a long-acting medication in patients who are unable to swallow slow-release morphine tablets whole, because its prolonged duration depends on delayed clearance, and it remains a long-acting agent when administered by elixir or crushed tablets. Methadone is inexpensive, but availability is limited in some pharmacies because of its historical association with opioid addiction treatment programs. Methadone's action as an NMDA antagonist may also increase its effectiveness in certain patients with neuropathic pain. Patients with neuropathic pain who do not respond well to dose escalation of other μ-agonists should be considered for a trial of methadone.

## Routes and Methods of Opioid Administration

### *Oral Administration*

Oral administration of drugs is the first choice for most patients. Oral dosing is generally predictable and inexpensive, and it does not require invasive procedures or technologies. Practical strategies for helping children with medications have been discussed elsewhere. [107] Oral dosing is not feasible in some children with severe unrelieved nausea or ileus or in the occasional child who is rendered uncooperative by extreme fear, delirium, or respiratory distress.

### *Intravenous Administration*

Intravenous administration has the advantage of rapid onset, titrated dosing, complete bioavailability, and constant effect when infusions are used. The main drawback is the need for intravenous access, which is limited in some patients but readily available in the large subgroup of pediatric oncology patients having central venous lines in place. Typical starting intravenous morphine infusion rates are 0.03 to 0.04 mg per kg per hour beyond the first 3 months of life, and 0.015 mg per kg per hour in younger infants.[108,109]

### *Subcutaneous Administration*

For children with poor intravenous access who require parenteral opioids, a convenient alternative is the use of continuous subcutaneous infusions of morphine or hydromorphone.[110] A small catheter or butterfly needle (27-gauge) may be placed under the skin of the thorax, abdomen, or thigh, the site being changed every 3 to 5 days, as needed. Solutions are concentrated so that infusion rates do not exceed 1 to 3 mL per hour, although some centers have used higher rates. The subcutaneous route can also be used for intermittent dosing or for PCA. [111] Needle placement can be made less noxious by prior use of EMLA cream. Pain at the injection site can be diminished by mixing one part lidocaine 1% (10 mg per milliliter) with nine parts opioid solution (lidocaine infusion rates should not exceed 1.5 mg per kg per hour). Intravenous and subcutaneous infusions can be made more convenient for the home by use of small portable infusion pumps. Most of these pumps are equipped with a PCA bolus option as well as a continuous infusion mode. Intramuscular dosing should be avoided in most circumstances, because it is painful and does not permit titrated dosing or infusion.

### *Patient-Controlled Analgesia*

PCA is a method of opioid administration involving a device, usually computer-controlled, that permits the patient to administer small bolus doses within set time limits. Table 43-2 outlines the advantages of PCA in pediatric patients. As noted earlier, PCA has been used very successfully for the management of prolonged oropharyngeal mucositis pain in adolescents. In one study, PCA and staff-controlled continuous-infusion morphine delivery were compared in a randomized, controlled trial in adolescents during oropharyngeal mucositis pain after bone marrow transplantation. The PCA group required less morphine, experienced less sedation, and had less difficulty concentrating, but they benefited from analgesia equivalent to that achieved by the group receiving staff-controlled continuous-infusion morphine.[37]

| |
|---|
| Permits titrated dosing to compensate for individual variation in pharmacokinetics or pharmacodynamics and pain intensity |
| Permits the patient to exercise control and diminish anxiety |
| Permits the patient to balance analgesia against side effects |
| Diminishes sedation |
| Is safe, well accepted |

**TABLE 43-2. ADVANTAGES OF PATIENT-CONTROLLED ANALGESIA IN PEDIATRIC PATIENTS**

PCA allows appropriately selected children control over their analgesia, which can be timed with routine mouth care and other causes of incidental mouth pain. It also allows children to choose a balance between the benefits of analgesia and the side effects of opioids. It is a standard of care at the Children's Hospital of Boston to institute patient-controlled opioid analgesia in appropriately selected children once topical measures and intermittent parenteral intravenous opioids have failed to produce adequate analgesia. In postoperative use, PCA is widely used successfully by children older than 6 years. Anecdotal experience suggests that some children between the ages of 4 and 6 years who become medically sophisticated during cancer treatment can use PCA successfully but, in this group, the risk of inadequate analgesia is higher, owing to failure of the patients to associate pain relief with pressing the button device on the PCA pump to administer opioid. We employ a basal infusion along with PCA-administered doses in most oncology patients.

### *Parent- and Nurse-Controlled Analgesia*

The use of parent-controlled analgesia or nurse-controlled analgesia has been somewhat more controversial. These two methods of analgesia administration permit caregivers to provide incremental, titrated dosing to patients who are unable to dose themselves, whether because of age, cognitive or motor impairment, or severe debilitation. The safety of PCA has been thought to derive from the fact that, when a patient becomes sedated, he or she stops pushing the PCA button device. When other caregivers are charged with pushing the button, this safety factor is removed. In practice, nurse-controlled analgesia has had a very good safety record and has been widely accepted in pediatric centers worldwide, largely for inpatients after surgery or for patients with cancer.

We make frequent use of parent-controlled analgesia in palliative care of children at home. In these circumstances, children generally are opioid-tolerant, which reduces the risk of hypoventilation, and parents have become experienced at judging their child's pain and clinical status. Conversely, parent-controlled analgesia has resulted in a significant number of cases of severe hypoventilation and several deaths among opioid-naive postoperative patients. In review of some of these cases, it appears that patients had risk factors that were inadequately appreciated, there was no formal program for parent education, and patient monitoring was sparse. In one published series of pediatric postoperative patients receiving either nurse-controlled analgesia or parent-controlled analgesia, hypoventilation requiring naloxone was not rare; because all patients were monitored, no adverse outcomes occurred.[112] Parents who will be operating a parent-controlled analgesia device should receive instruction in the aspects of pain assessment, the effects of opioids, and dose titration. They should have a support system that permits immediate telephone availability of clinicians with whom they can confer about dosing. Because in most cases the terminally ill child ultimately will die at home, parents must be reassured in advance that the cancer, not their dosing of opioids to allow comfort, is the cause of their child's demise.

### Opioid Dose Escalation, Opioid Switching, and Incomplete Cross-Tolerance

The effective opioid dose for treatment of cancer pain is extremely variable, and dosing should be titrated based on analgesia and side effects. The WHO analgesic ladder (Fig. 43-1) presents an algorithm for the prescription of analgesics in the setting of cancer pain, in which analgesia is prescribed in a step-wise manner, ranging from simple analgesics for mild pain to strong opioids for intense pain.

*Tolerance* refers to the progressive decline in potency of an opioid with continued use, such that increasingly higher doses are required to achieve the same analgesic effect. When tolerance to a particular opioid develops, cross-tolerance to other opioids may be incomplete. *Physical dependence* is a physiologic state characterized by withdrawal (i.e., abstinence syndrome) after discontinuation of the opioid. Initial manifestations of withdrawal include yawning, diaphoresis, lacrimation, coryza, and tachycardia. Addiction is a psychological and behavioral syndrome characterized by drug craving and aberrant drug use.

Patients and parents often are reluctant to increase dosing because of a fear that tolerance will make opioids ineffective at a later date. They should be reassured that tolerance in the great majority of cases can be managed by simple dose escalation, use of adjuvant medications, or perhaps by opioid switching in the setting of dose-limiting side effects. No justification exists for withholding opioids to save them for a later time of need. Some parents may fear that administration of opioids to their child will cause the child subsequently to become a drug addict. The incidence of opioid addiction was examined prospectively in 12,000 hospitalized adult patients who received at least one strong opioid. Only four cases of subsequent addiction in patients without a prior history of drug abuse were documented. These data suggest that iatrogenic opioid addiction is an exceedingly uncommon problem.[113]

Traditionally, opioids are regarded as interchangeable in terms of the development of tolerance and the ratio of analgesia to side effects. Cases were described that seemed to exhibit two novel features: incomplete cross-tolerance when switching from one opioid to another, and markedly different efficacy or ratio of analgesia to side effects with different opioids.[114] These phenomena warrant further study. If intolerable side effects are found with dose escalation of one opioid, it is reasonable to try another opioid. Foley[115] recommended reducing to 50% of the equianalgesic dose when switching from an opioid with a short half-life to another in the same category. As noted earlier, transition from another opioid to methadone in particular should be undertaken with caution, and even lower starting doses of methadone should be used.[106] Patients who have been switched from a short half-life opioid to a long half-life opioid require close observation. The development of progressive somnolence may indicate the need to reduce the dose or frequency of administration. Table 43-1 demonstrates the potency of the various opioids relative to morphine. Clinical examples of opioid dose calculation and conversion for treating pediatric cancer pain are given in Table 43-3.

**TABLE 43-3. CLINICAL EXAMPLES OF OPIOID DOSE CALCULATION AND CONVERSION FOR PEDIATRIC CANCER PAIN**

### *Pain Associated with Advanced Cancer in Childhood*

Among adults with cancer, rapid opioid dose escalation is most commonly attributed to tumor spread rather than rapidly progressive tolerance.[116] The opioid requirements and dose escalation were examined for a cohort of 199 children who died of malignancy at Boston's Children's Hospital and the Dana-Farber Cancer

Institute from 1989 to 1993, a time during which the WHO program was more consistently applied. [31] Twelve patients(6%) required greater than 100-fold escalation over starting values or greater than 3 mg per kg per hour of intravenous morphine equivalent. This rapid dose escalation occurred most commonly in the final weeks of life (Fig. 43-2) and among patients with solid tumors metastatic to the spine, central nervous system, or major nerve plexus. Maximum opioid dosing ranged from 3.8 to 518 mg per kg per hour of intravenous morphine equivalent. The maximum infusion rate (exceeding all previously published reports) occurred in an infant with an isolated metastasis in the periaqueductal gray matter, a brainstem site linked to mediating analgesia and defense reactions. [117]

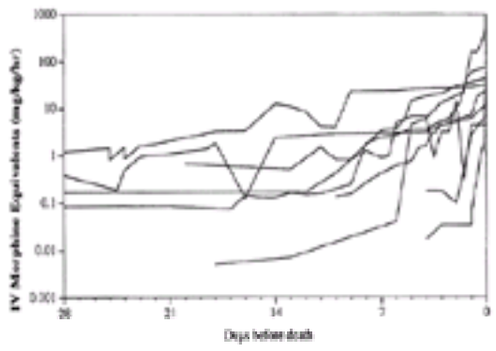


**FIGURE 43-2.** Logarithmic escalation of dose for pediatric patients with terminal malignancies who require massive doses of opioids. (From Collins JJ, Grier HE, Kinney HC, Berde CB. Control of severe pain in children with terminal malignancy. J Pediatr 1995;126:653, with permission.)

Standard dosing of opioids adequately treats most cancer pain in children; however, a significant number of pain episodes related to cancer require more extensive management. Of the 12 patients in our series, [31] eight required extraordinary measures (i.e., epidural or subarachnoid infusion and sedation) to achieve adequate analgesia. The clinical course of care derived from the management of these patients with intractable pain during the terminal phase of their illness is shown in Figure 43-3.

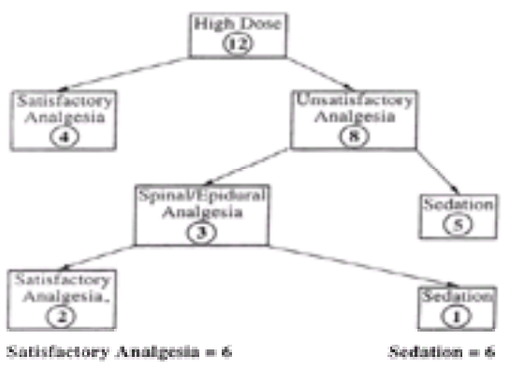


**FIGURE 43-3.** The clinical course of 12 pediatric patients requiring high-dose opioid therapy for terminal malignancies. Circled numbers represent the number of patients in each category. Ultimately, analgesia was satisfactory in six patients. (From Collins JJ, Grier HE, Kinney HC, Berde CB. Control of severe pain in children with terminal malignancy. J Pediatr 1995;126:653, with permission.)

The choice of sedation as a method of analgesia generally assumes that there is no acceptable means for providing analgesia with preservation of alertness. We strongly recommend continuing high-dose opioid infusions along with sedative-hypnotics in these circumstances, to avoid situations in which the patient has unrelieved pain but inadequate clarity of sensorium to communicate about his or her pain. The ethics of providing sedation in the terminally ill is discussed elsewhere.[118] A retrospective survey of parents after the death of their child due to cancer indicated that a high percentage of children experienced delays or incomplete effectiveness in achieving relief of pain and other symptoms. [119]

### *Treatment of Opioid Side Effects*

All opioids can potentially cause the same constellation of side effects. However, the constellation experienced by individual patients receiving different opioids may not be the same. Children do not necessarily report all side effects (e.g., constipation, pruritus) and should be asked specific questions. The assessment of analgesic effectiveness includes an assessment of opioid side effects. Tolerance to sedation, nausea and vomiting, and pruritus often develop within the first week after commencing opioids. One uncommon side effect of opioids is the onset of dreams that usually are described as vivid in quality, although not necessarily frightening. A change of opioid may be required if this is distressing to the child. Table 43-4 outlines management strategies for common opioid side effects.

**TABLE 43-4. MANAGEMENT OF OPIOID SIDE EFFECTS**

## Invasive Approaches to Pain Management

### *Anesthetic Approaches*

Several techniques are used extensively for adult cancer patients who experience inadequate relief with massive dose escalation or who experience intolerable side effects with the use of opioids and adjuvant medications. The most widespread invasive technique for adults in the United States involves epidural or subarachnoid infusions of opioids and local anesthetics. [120],[121] and [122] Experience with children is much more limited, but preliminary experience in several centers suggests that selected use of a spinal infusion may be helpful.

Based on experience with epidural and subarachnoid infusions among 12 children and adolescents with cancer, [123] we recommend the following modifications of

technique for children relative to common adult practice:

- Adults commonly receive placement of epidural and spinal catheters while they are awake or with minimal sedation. This is an entirely unnecessary source of distress, and we advocate placement of epidural and subarachnoid catheters in children under effective sedation or general anesthesia. Positioning of catheters should be confirmed by fluoroscopy and contrast injection at the time of placement, whenever feasible, to verify proper positioning and to confirm that the access of medications to the intended sites of action is not occluded by spinal metastases.
- Although many adults obtain an adequate ratio of analgesia to side effects with opioids alone, children and adolescents invariably require some local anesthetic along with an opioid to achieve optimal effects. Morphine, fentanyl, and hydromorphone have commonly been used via the epidural route in children.
- Catheters should be tunneled at the time of initial placement for improved skin care and maintenance.
- Because local anesthetics usually are needed and their cephalocaudad spread is limited, particularly by the epidural route, optimal placement of catheters is at or above the appropriate dermatomal level of the patient's major sites of pain. For pain predominantly above the umbilicus, we prefer placement of thoracic epidural catheters. For pain predominantly below the umbilicus, we prefer lumbar subarachnoid catheters to give the greatest flexibility in local anesthetic dose escalation.
- With spinal analgesia, particularly using local anesthetics, requirements for systemic analgesia vary widely. Patients may become sedated from minimal systemic opioids because of the reduction of afferent stimuli, or they may experience withdrawal from rapid reduction of systemic opioids. Dosing must be individualized on the basis of clinical signs.
- Maintaining a route for rescue medication, including intravenous boluses, and for planning of dose escalation of spinal medications is essential. Some patients will need additional systemic opioid analgesia or anxiolytics for the management of terminal air hunger.

Neurolytic blockade using agents such as phenol or alcohol is used most widely for adults with visceral malignancy, such as celiac plexus blockade for pancreatic cancer[124] or hypogastric plexus blockade for pelvic malignancies.[125] Neurolytic blockade is used rarely in children, although we found celiac plexus blockade useful for a child with hepatoblastoma that was unresponsive to treatment.[126] Specialists with experience in pediatric regional anesthesia and cancer pain management should be consulted if anesthetic techniques are considered for children with widespread cancer pain.

### *Neurosurgical Approaches*

The use of neurodestructive procedures in adults with cancer pain has diminished as our facility with systemic opioids and adjuvants has improved and as spinal infusion of opioids and local anesthetics has become available. Experience with neurodestructive procedures in children is extremely limited. In an earlier era when opioids were used less effectively, Matson[127] described his experience with effective use of cordotomy in children, but whether these cases could have been managed effectively by current pharmacologic approaches remains unclear.

A discussion of the considerations for neurosurgical approaches to pain in children is given elsewhere.[128] These authors describe several cases of decompressive laminectomies that relieved refractory pain for patients with acute cord compression. Considerations for surgery, irradiation, or medical therapy of cord compression must be individualized. An algorithm for the management of back pain and spinal cord compression in adults has been proposed by Portenoy.[129]

The choice of invasive methods of pain management in patients with terminal malignancy should be made judiciously, with a consideration of the wishes of the patient and family. For example, if opioid escalation is limited by somnolence or confusion despite addition of a stimulant, a primary consideration is whether the patient wishes to remain alert. Some children prefer somnolence during their terminal course. In this situation, escalation of opioids and anxiolytics may be preferable to an invasive procedure. Other children wish very strongly to have alertness along with comfort, and an invasive method of analgesia may then be appropriate.

## Adjuvant Agents

The term *adjuvant analgesic* describes a heterogeneous group of drugs that have a primary indication other than pain but are analgesic in some painful conditions.[130] Common classes of drugs that have been used as adjuvant analgesics include antidepressants, anticonvulsants, neuroleptics, psychostimulants, antihistamines, corticosteroids, centrally acting skeletal muscle relaxants, and associated drugs. These agents are commonly, but not always, prescribed with primary analgesic drugs.

### *Antidepressants*

The use of tricyclic antidepressants for the treatment of chronic pain in adult patients was first reported in 1960.[131] Subsequently, tricyclic antidepressants have been used for a variety of pain conditions, including post-herpetic neuralgia,[51] diabetic neuropathy,[132] tension headache,[133] migraine headache,[134] rheumatoid arthritis,[135] chronic low back pain,[136] and cancer pain.[137] Tricyclic antidepressants may be useful as an adjuvant analgesic in patients with neuropathic pain caused by tumor. The mechanism of action of tricyclic antidepressants as analgesics and the method by which they may potentiate morphine analgesia have been reviewed elsewhere.[138,139]

Despite the lack of controlled trials on the use of tricyclic antidepressant drugs as adjuvant analgesic agents in the unwell pediatric population, guidelines for the choice and management of antidepressants as adjuvant analgesics in children have been outlined by Heiligenstein and Gerrity.[140] These guidelines include the premise that

- It is reasonable to prescribe when shown to be effective in adults.
- NSAIDs or opioids should be tried first, except in deafferentation pain.
- The choice usually is made on the basis of side effect profile (in patients with insomnia and pain, consider a sedating compound at bedtime).
- A baseline complete blood count, electrolyte assay, liver enzyme profile, and electrocardiogram (to exclude Wolff-Parkinson-White syndrome or other cardiac conduction defect) should be performed.[141]

Because children metabolize these agents more efficiently than do adults, some psychiatrists employ a twice-daily regimen of tricyclic antidepressants, often with a larger dose at night than in the morning. Our preference for most ambulatory patients is to use only nighttime dosing. Indications for blood levels include confirmation of compliance and confirmation that optimization of dosage has occurred before the drug's use is discontinued. A titration electrocardiogram is recommended for long-term use or if standard dosages are exceeded, as asymptomatic electrocardiographic changes can occur.[142]

The anticholinergic and sedating side effects of amitriptyline may be a significant problem for some patients. A change to a tricyclic drug with fewer sedating or anticholinergic side effects (e.g., nortriptyline, desipramine) is an option. Amitriptyline is available in a parenteral preparation, and use of this route of administration in children has been described elsewhere.[143]

Common causes for insomnia in children with cancer include unrelieved pain, anxiety, and depression. If pain is a cause of sleep disturbance, a prescription of a larger dose of opioid at bedtime or the use of sustained-release preparations (e.g., MS-Contin) or slowly metabolized opioids (e.g., methadone, levorphanol) can diminish the chance of nighttime awakening from pain. Otherwise, our preference is to treat most sleep disturbances with small doses of a tricyclic antidepressant or with the tetracyclic antidepressant trazodone. We discourage the prolonged use of benzodiazepines for sleep disturbance, because with chronic use, they disrupt sleep cycles, produce tolerance and dependence, and can exacerbate daytime somnolence and confusion.

### *Psychostimulants*

The analgesic effects of amphetamines are believed to be mediated by central and descending spinal inhibitory pathways. Descending noradrenergic and serotoninergic spinal inhibitory pathways may play a role in the analgesic action of methylphenidate.[144] Psycho stimulants have multiple potential benefits as adjuvant drugs in pain management. Dextroamphetamine potentiates opioid analgesia in postoperative adult patients.[145] Methylphenidate counteracts opiod-related sedation[146] and cognitive dysfunction[147] in advanced cancer patients and may allow dose escalation of opioids in cancer patients who have somnolence as an opioid-limiting side effect.[148] Yee and Berde[149] reported on the safety, efficacy, and tolerability of dextroamphetamine and methylphenidate in a retrospective review of 11 children receiving opioid for a variety of indications, including cancer pain. Somnolence was reduced without significant adverse side effects.[149] Prescription of methylphenidate should be preceded by consideration of its potential side effects, which include anorexia, insomnia, and dysphoria.

### *Corticosteroids*

Corticosteroids mediate their analgesic activity in the setting of cancer pain by a variety of mechanisms, including antiinflammatory effects, reduction of tumor edema and, potentially, reduction of spontaneous discharge in the injured nerve. [150] Most studies examining the role of steroids in the setting of cancer pain have evaluated adults. Steroids have a role to play in the setting of bone pain due to metastatic bone disease, [151] cerebral edema due to primary or metastatic tumor,[152,153] epidural spinal cord compression[154] and, possibly, neuropathic pain. [155] Although the optimal type and dosage of corticosteroids for various indications have not been adequately studied, dexamethasone often is used because of its high potency, longer duration of action, and minimal mineralocorticoid effect.

### *Anticonvulsants*

Anticonvulsants are effective for many forms of neuropathic pain. Although traditionally used for lancinating or paroxysmal neuropathic pain, they also are useful for many forms of constant burning pain or for conditions with severe allodynia. [156] Carbamazepine has been used effectively for lancinating pain due to cancer. Phenytoin, clonazepam, and valproate may have similar effects. The mechanism of action of anticonvulsants in controlling lancinating pain is unknown but is presumed to be related to reducing paroxysmal discharges of central and peripheral neurons. [157]

In recent years, gabapentin has emerged as the most commonly used anticonvulsant for neuropathic pain. In comparison with the agents listed earlier, this anticonvulsant is associated with a reduced risk of hematologic, hepatic, or autoimmune complications. Controlled trials have demonstrated analgesic effects in adults with diabetic neuropathy and post-herpetic neuralgia. The dose response and maximum tolerated dose of gabapentin vary widely. Our general approach is to begin with a first day of very low doses administered twice daily (e.g., 50-mg morning dose and 100-mg evening dose for adolescents; 25- and 50-mg doses for younger children). (The smallest capsules are 100 mg, so the contents are dissolved in juice and a fraction of the volume is administered.) If the drug is tolerated, dosing is continued on a three-times-daily schedule with larger evening doses than daytime doses, and is escalated on a daily basis by 50% until one of three end points is reached: (a) analgesia is achieved, (b) side effects (sedation, changes in memory or cognition, mood changes) become bothersome, or (c) dosing reaches roughly 50 to 60 mg per kg per day.

A number of other new anticonvulsants are under investigation for neuropathic pain, including topiramate, lamotrigine, and pregabalin. Information on pediatric use of these agents is limited, and their use has largely been confined to treatment of refractory epilepsy.

### *Neuroleptics*

Phenothiazines and butyrophenones are largely indicated as antiemetics rather than as analgesics. In some cases, neuroleptics given to patients in pain may reduce their ability to interact and verbalize their pain rather than reduce the intensity of the pain experience. Evidence for an analgesic effect for most of the neuroleptics is scant.

The phenothiazine methotrimeprazine appears to be analgesic in the setting of adult cancer pain. [158] It may be useful as an adjuvant analgesic in the patient with advanced cancer should not be considered a substitute for opioid analgesia. The mechanism by which methotrimeprazine produces analgesia and its role as an adjuvant agent in pediatric cancer pain are unclear.

### *Radionuclides*

One case report indicated the potential role of [ $^{131}$I]-meta-iodobenylguanidine ([ $^{131}$I]MIBG) in painful bone disease related to disseminated neuroblastoma. [159] Administration of [$^{131}$I]MIBG occurred on three separate occasions and allowed cessation of opioids subsequent to the administration of this agent. The side effects of [$^{131}$I]MIBG were thrombocytopenia and cystitis. The use of other radionuclides for painful metastatic bone disease in adults has been reported. [160]

## NAUSEA AND VOMITING

The intensification of both chemotherapeutic and radiotherapeutic programs has resulted in both increased efficacy in and increased toxicity to cancer patients. Nausea and vomiting, which are experienced by almost all cancer patients, are among the most troublesome and debilitating side effects. Nausea (the feeling of the imminent need to vomit) and vomiting (the forceful expulsion of gastric contents) are not the self-limiting symptoms commonly associated with other disease states. Rather, they are severe and often prolonged symptoms associated with numerous unpleasant sequelae ranging from wound dehiscence to dehydration that necessitates hospitalization. Without effective prophylaxis, these symptoms become debilitating, and patients are physically incapable of receiving further chemotherapy or are so psychologically distressed that they or their parents may refuse subsequent treatments.

During the last two decades, major advances have led to better, although still imperfect, control of nausea and emesis. These advances stem from improved understanding of the physiology of nausea and vomiting, the development of the 5-hydroxytryptamine subtype 3 (5-HT $_3$) receptor antagonist class of antiemetics, and improved differentiation of anticipatory, acute, and delayed symptoms. Despite these advances, nausea and vomiting remain the first and third most distressing side effects of chemotherapy in adults and continue to occur in most adult and pediatric patients. [161,162 and 163]

### Physiology of Vomiting

Vomiting is mediated by the vomiting center located in the medullary lateral reticular formation. [164] This center receives afferent input from five main sources:

1. Chemoreceptor trigger zone (CTZ)
2. Vagal and sympathetic afferents from the viscera
3. Midbrain receptors that detect changes in intracranial pressure
4. Labyrinthine apparatus, which detects motion and position
5. Higher central nervous system structures (e.g., the limbic system)

The vomiting center, in turn, activates a series of efferent pathways, which include phrenic nerves to the diaphragm, spinal nerves to abdominal musculature, and visceral nerves to the stomach and esophagus. These efferent pathways respond to the centrally mediated stimulation in the vomiting center and act to induce actual vomiting.

### *Chemotherapy-Induced Vomiting*

Chemotherapeutic agents may induce vomiting either by stimulation of the vomiting center itself or via direct or indirect stimulation of the CTZ. The CTZ, a distinct medullary center located in the floor of the fourth ventricle in the vicinity of the area postrema, activates the vomiting center to produce nausea and vomiting. The CTZ has no autonomous capability to produce vomiting.

Animal studies of CTZ ablation suggest that chemotherapeutic agents induce nausea and vomiting by CTZ stimulation. [165] Participation of the forebrain and peripheral mechanisms has also been demonstrated.[166] However, major species differences exist. Thus, one can only infer the site of action in human beings by extrapolation from animal studies and by clinical observations. Several findings suggest that the action of chemotherapeutic agents on the CTZ is indirect: First, it appears unlikely that the CTZ would have a unique receptor for each drug. Second, vagotomy and sympathectomy abolish cisplatin-induced vomiting in the ferret. [167] Third, transfer of whole plasma, plasma filtrates, and blood from dogs and cats with cisplatin-induced vomiting fails to induce vomiting in the recipient. [168] Current evidence suggests that neuronal afferents activated by chemotherapeutic agents increase neural input to the CTZ. This hypothesis is supported by animal experiments in which denervation of the vagal and sympathetic afferents prevents chemotherapy- and radiation-induced emesis. [166,167] Whether chemotherapeutic agents act on the gastrointestinal tract wall to release a humoral or neuronal signal or act directly on vagal afferents is under investigation.

Our understanding of the neurochemistry of vomiting has improved but remains incomplete. [169] We now know that serotonin (5-HT) receptors, particularly subtype 3

receptors (5-HT$_3$), play a role in the mediation of chemotherapy-induced nausea and vomiting. Selective antagonists of 5-HT $_3$ receptors are potent antiemetics. Enterochromaffin cells in the gastrointestinal tract are major producers of serotonin, and studies in ferrets have demonstrated that cisplatin-induced emesis can be prevented with depletion of body serotonin stores.[170] Human studies have shown a rise in serotonin metabolites in the urine after administration of cisplatin.[171] This rise correlates with the onset and intensity of emesis. 5-HT $_3$ receptors are located throughout the human brain, including a high concentration in the area postrema, where the CTZ is located.[172] Injection of 5-HT$_3$ receptor antagonists into the area postrema inhibits cisplatin-induced emesis in the ferret.[173] 5-HT$_3$ receptors are present in vagal afferent fibers, and activation by serotonin leads to an increased firing rate in these fibers.[174] In addition, metoclopramide, which previously was known to act on dopaminergic receptors, now has been shown to act on serotonin receptors as well.[175] Whether the predominant clinical effect of 5-HT $_3$ receptor antagonists is central, on receptors in the area postrema, or peripheral, on receptors in the gastrointestinal tract wall or vagal afferents, remains unclear.

Recent work suggests that substance P, a neuropeptide found in the gastrointestinal tract and central nervous system, may play a role in mediating emesis from a number of stimuli. Substance P antagonists, which antagonize the neurokinin-1(NK $_1$) receptor, inhibit vomiting in animals from a number of stimuli, including radiation and chemotherapy.[176,177,178,179,180 and 181] These NK$_1$ receptor antagonists are effective antiemetics when administered with the emetogenic treatment or when administered after the first emesis has occurred.[179,181] In ferrets, regular administration of an NK$_1$ antagonist inhibits vomiting after cisplatin administration more effectively than does ondansetron.[182] Hence, in the near future, NK$_1$ receptor antagonists may be developed as effective antiemetics, particularly for delayed emesis.[183,184,185 and 186]

### *Radiation-Induced Vomiting*

Radiation-induced nausea and vomiting appear to be mediated through both CTZ and peripheral mechanisms.[187] Total-body, cranial, and abdominal radiation are all emetogenic, particularly the latter.[188,189 and 190] The role of serotonin in emesis induction is suggested by the findings that higher urine serotonin metabolite levels have been correlated with more emetogenic radiation and that 5-HT $_3$ receptor antagonists are effective in controlling radiation-induced emesis.[190,191]

### *Disease-Induced Vomiting*

Nausea and vomiting result from various sequelae of cancer. Metastatic disease may produce tumor exudate or sloughing of tissue, which in turn produces toxic central effects. Abnormally high or low intracranial pressure, stretching of the capsule of an organ, inflammation of the gastrointestinal tract (gastritis or gastroenteritis), or gastrointestinal obstruction may also initiate nausea and vomiting. The exact mechanisms of symptoms are not clearly defined for these states. However, some of these states can respond to both surgical and pharmacologic intervention. Opioid-induced vomiting and opioid-induced ileus leading to vomiting are common among patients at all stages of illness. Opioids produce nausea and vomiting by both brainstem and direct enteral afferent mechanisms.

## Clinical Presentation

Great variability occurs in symptom presentation, intensity, time to onset, and duration. Some of this variability can be predicted from the specific treatment modalities used, but great variability among patients in response to identical regimens also exists. Symptoms include nausea, retching, and vomiting and tend to occur in a cyclic fashion if not properly treated early in therapy. Retching is identical to vomiting in that the same physiologic mechanisms are occurring, but gastric contents are not expelled. Clinically, retching often is perceived by the patient to be more debilitating than vomiting, because the abdominal musculature can be significantly strained and no relief is achieved. *Acute* symptoms occur during the first 24 hours after administration of chemotherapy. *Delayed* nausea and vomiting occur 24 to 120 hours after emetogenic treatment. Almost all patients experience delayed symptoms after cisplatin administration; nearly half do so after moderately emetogenic treatment.[192,193 and 194] Three-fourths of adult patients experience delayed emesis on the third to fourth days after cisplatin administration.[195] *Anticipatory (psychogenic) vomiting*, the onset of nausea and vomiting before the administration of chemotherapy, is difficult to treat because it is a conditioned response and may be related to anxiety.[196,197] Despite major improvements in the management of acute and delayed symptoms, more than one-half of pediatric patients experience anticipatory symptoms.[196,198] Patient factors that increase the probability of severe clinical symptoms in adults include a prior chemotherapeutic experience, a predisposition to nausea and vomiting (e.g., motion sickness), anxiety, and being female.[199] A multivariate analysis found that prechemotherapy nausea, low social functioning, and female gender were extremely predictive of an increased incidence of symptoms.[200] Better control of acute nausea and vomiting is associated with a lower incidence of delayed nausea and vomiting.[201,202] Of note, pediatric studies addressing risk factors are very limited. One pediatric study does suggest that girls have more symptoms than boys and children younger than 6 years may demonstrate fewer symptoms.[203]

## Principles of Therapy

The origin of vomiting must be identified before any therapy is initiated. In addition to chemotherapy, other causes of vomiting common to pediatric cancer patients include stretching of the capsule of an organ, vestibular reflexes (motion sickness), inflammation of the gastrointestinal tract (gastritis, gastroenteritis), gastrointestinal obstruction, increased intracranial pressure, narcotic administration, and ileus. The latter may be induced by chemotherapeutic agents that affect the peripheral nervous system (e.g., vincristine), by narcotics, or by direct gastrointestinal tract damage from disease or treatment. The etiology of vomiting may change with the patient's condition. For example, the same chemotherapy that directly caused acute and delayed symptoms may then cause gastrointestinal inflammation, leading to continued vomiting. Although treatment-induced symptoms generally are predictable, unusual severity, timing, or duration should prompt development of a differential diagnosis, not just a reflexive modification of the antiemetic regimen. Such symptoms could be the manifestation of a completely different process, such as intestinal obstruction or increased intracranial pressure.

To prevent treatment-induced nausea and vomiting, the receptors for the emetic stimulus must be blocked before the stimulus occurs and the blockade must be continued as long as symptoms are likely to occur. If the emetic stimuli are noniatrogenic (e.g., infection or metabolic derangement), therapy cannot be initiated before symptoms become established. Because nausea and vomiting in patients receiving chemotherapy or radiotherapy are predictable, a planned approach to prophylactic antiemetic therapy is indicated. Scheduled doses must be administered in a timely fashion, regardless of whether symptoms appear. The duration of follow-up therapy is determined by both the patient's previous patterns of nausea and vomiting and the expected duration of emetic activity of the stimulus. Intravenous therapy was once the standard of care for all patients. Newly available oral agents offer good bioavailability and marked efficacy in adults but have not been studied in children.[204,205,206,207 and 208]

Knowledge of the emetogenic potential and patterns of vomiting associated with the various chemotherapeutic agents helps to predict the severity and duration of the anticipated symptoms. Table 43-5 lists some of the antineoplastic agents most commonly used in children, categorized by the severity of symptoms. Data addressing potential differences in emetogenicity between adults and children are sparse. Some agents characterized as most emetogenic in adults have been reported as mildly emetogenic in children.[209,210 and 211] Triple intrathecal therapy with methotrexate, cytarabine, and hydrocortisone has been shown to be moderately emetogenic in children, but the effect of each drug alone has not been addressed.[212] The variability in acute and delayed emetogenic potential of these agents is great. The agents at the lower end of the scale may induce moderate nausea accompanied by no vomiting or mild vomiting, whereas those at the upper end may produce debilitating nausea accompanied by severe vomiting. The severity and duration of vomiting also differ among patients. Some patients may experience 20 retching or vomiting episodes each day for 5 days, whereas others may have 50 episodes over a span of a few hours. The time of onset also varies. Acute symptoms may appear immediately (methchlorethamine) or 6 to 12 hours after administration (cyclophosphamide or actinomycin-D). Delayed symptoms do not appear until at least 24 hours after administration. When these agents are used in combination, antiemetic prophylaxis should be based on the most emetic component of the regimen. When combinations of moderately emetogenic agents are used, prophylaxis for severely emetogenic treatment should be considered. The severity of nausea and vomiting usually is increased with increasing dose and decreased with increasing infusion time.[209] Increasing use of multiday chemotherapeutic regimens, recognition of delayed vomiting, and availability of effective antiemetics with few side effects have led to longer administration of antiemetics. Nausea and vomiting associated with radiotherapy are less well understood. Abdominal radiation almost always causes nausea and vomiting, whereas the occurrence and severity of nausea and vomiting from cranial radiation are more variable.

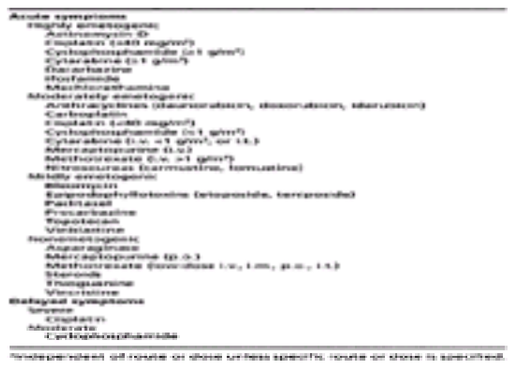

**TABLE 43-5. EMETOGENIC POTENTIAL OF CHEMOTHERAPEUTIC AGENTS**[a]

Additional factors affect the success of the antiemetic regimen chosen. The efficacy of placebo and the negative effects of the vomiting roommate demonstrate the importance of suggestion.[213] Pretreatment anxiety and taste of drugs during injection can also predict symptom development.[214] Hypnosis and supportive counseling can decrease symptoms.[215,216] Removal of known stimuli such as the sight or smell of food also can decrease symptom occurrence. In addition, individual patient preferences regarding oral versus parenteral and soporific versus nonsoporific therapy must be considered. Sleep itself may be used as an antiemetic agent. [217]

### *Pharmacologic Approaches to Control of Acute Symptoms*

Drugs used in the prevention of nausea and vomiting include both true antiemetics and ancillary agents. The latter are not always true antiemetics but are used to potentiate the effects of true antiemetics, to treat anxiety, or to induce sleep. True antiemetics can be classified on the basis of their therapeutic index, which accounts for both efficacy and side effects. Side effects include not only such obvious problems as extrapyramidal symptoms and hallucinations but more subtle symptoms such as dysphoria or soporific effects. Because the vast majority of the antiemetic literature, including most randomized, controlled antiemetic studies, have included only adult patients, we are forced to extrapolate from the adult literature approaches to antiemetic management in children. [210] These studies do not account for potential differences between adults and children as regards antiemetic half-life or side effects or in terms of the emetogenic potential of various drugs.

In pediatric patients, route of administration and available dose forms can greatly affect the efficacy of an agent. Developmental or psychological reasons may prevent administration of oral tablets or capsules in some children. For other children, the only available oral or transdermal dose forms may be inappropriately high. Thus, clinical practice guidelines developed in the adult setting may not be fully applicable to children. [210,218]

Although visual analog scales are used routinely to measure nausea in adults, application of such scales to children is difficult. [219] Interpretation of the pediatric literature is often limited by lack of randomization, heterogeneity of the patient population, and heterogeneity of the emetogenic regimens. Specific dosing parameters are usually unavailable for children because these agents have not received full clinical evaluation in the pediatric population. Table 43-6 outlines various antiemetic agents in the order in which they are discussed. The dosage recommendations presented are the result of our clinical experience and should serve only as a guideline for establishing proper antiemetic regimens.

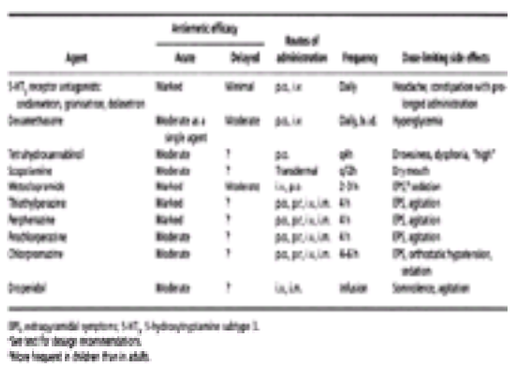

**TABLE 43-6. ANTIEMETIC AGENTS**[a]

### *5-HT$_3$ Receptor Antagonists*

The 5-HT$_3$ receptor antagonists are potent antiemetics with a wide therapeutic margin. Their development in the 1980s and widespread use in the 1990s has revolutionized the prevention of nausea and vomiting in cancer patients. Prior to the availability of these agents, the efficacy of the most widely used antiemetics, metoclopramide and the phenothiazines, was severely limited by their extrapyramidal side effects. Initial research on the different 5-HT $_3$ receptor antagonists focused on each individual agent to determine appropriate dosing, efficacy as compared to metoclopramide and phenothiazines, and optimal use of concomitant agents. More recent research has shown a threshold effect but no dose response, relative equivalence of oral and parenteral routes, and remarkable therapeutic and toxic equivalence between the different 5-HT$_3$ receptor antagonists.[206,208,210] Their role in the control of delayed symptoms is unclear at best.[220,221,222,223,224,225] and [226] Because these agents are costly, development of clinical practice guidelines is encouraged to ensure their appropriate use. [227]

Ondansetron was the first 5-HT$_3$ receptor antagonist commercially available in the United States. For control of acute symptoms in adults, ondansetron is highly effective[171,228,229,230,231] and [232] and superior to metoclopramide.[233,234,235,236,237] and [238] Its efficacy is enhanced by the addition of dexamethasone.[239] The majority of the early studies in adults used a three-dose intravenous regimen of 0.15 mg per kg per dose or 8 mg per dose, with or without subsequent oral maintenance. Subsequent adult studies have shown that single-dose regimens of 16 to 32 mg are at least as effective as divided dose regimens and benefit from the addition of dexamethasone.[224,240,241] Oral regimens offer good bioavailability and have been shown to be effective for moderately and highly emetogenic chemotherapy but have not been studied as extensively as intravenous therapy. [204,242,243] Toxicityin most studies has been minimal and consists primarily of headache (10% to 15%), transient elevation of hepatic transaminases (6% to 8%), and constipation (5%).

Nonrandomized pediatric studies have shown ondansetron to be effective in children receiving a variety of chemotherapeutic agents and radiation.[211,212,244,245,246,247,248,249,250] and [251] Randomized pediatric studies with ondansetron have shown better efficacy compared to metoclopramide, that doses greater than 5 mg per m$^2$ do not add to efficacy, and that the addition of dexamethasone improves efficacy.[252,253,254] and [255] Some pediatric studies show a lower incidence of headache (<5%) and constipation (<1%) than in adults.[247,255,256] Although pharmacokinetic studies in adults have shown trends to correlations between antiemetic efficacy and the area under the curve, the clinical impact of the shorter half-life of ondansetron in children as compared to adults is unknown. [251,257,258]

Few data regarding ondansetron dosing in children are available. Most published studies have used divided-dose regimens with three daily doses of 0.15 mg per kg per dose or 5 mg per m$^2$ per dose. Oral ondansetron twice daily has been given after the initial intravenous drug in many studies. A two-dose regimen of 0.15 mg per kg has been effective in mildly and moderately emetogenic chemotherapy.[211] When ondansetron first became available in the United States, our clinical practice was to administer 0.15 mg per kg per dose before and 8 and 16 hours after chemotherapy. For convenience and cost savings, we have extrapolated from adult data to arrive at our usual ondansetron dose of 0.45 mg per kg per day given intravenously as a single dose.

Granisetron, the next 5-HT$_3$ receptor antagonist to become commercially available in the United States, usually is administered as a single intravenous or oral dose

prior to the administration of emetogenic chemotherapy. Conflicting data appeared in earlier studies regarding the optimal dose, schedule, and route of administration.[195,259,260] Current adult consensus guidelines and recent studies use a dose of 2 mg by mouth daily. For the control of acute symptoms, the combination of dexamethasone and granisetron is more effective than granisetron alone.[223,261] Limited pediatric granisetron data are consistent with adult data in terms of efficacy.[163,262,263,264,265 and 266] The appropriate pediatric dosage of granisetron is unclear at present. Despite the manufacturer's recommended dose of 10 mcg per kg, at least one study has shown lack of efficacy at that dose.[267] Data regarding the relative efficacy of 20 mcg per kg and 40 mcg per kg are conflicting.[203,268] Data regarding the use of oral granisetron in children are not found in the literature.

Dolasetron, the 5-$HT_3$ receptor antagonist most recently available in the United States, is usually administered as a single intravenous or oral dose of 100 mg or 1.8 mg per kg.[269,270,271 and 272] Pediatric studies with small numbers of patients suggested the possibility of increased efficacy with a single dose of 1.8 mg per kg given either intravenously or orally.[273,274] This same group found a shorter half-life for the active metabolite of dolasetron in younger children but studied an insufficient number of patients to determine the clinical significance of this finding.

Tropisetron, a 5-$HT_3$ receptor antagonist that is not currently available in the United States, is characterized by excellent oral absorption, a high area under the curve after a single dose, and few side effects.[275] Dose-finding studies suggest that a single daily dose of 5 mg is adequate in adults.[276] Efficacy as a single agent is good[277,278] and, as is true for all 5-$HT_3$ receptor antagonists, its efficacy is increased with the addition of dexamethasone.[278,279 and 280] Doses of 0.20 mg per kg, 5 mg per $m^2$, and 2 to 5 mg per day have been shown to be effective and well tolerated in children receiving various chemotherapeutic regimens.[281,282 and 283]

On the basis of the published literature, finding an efficacy or toxicity rationale to support the use of one 5-$HT_3$ receptor antagonist as opposed to another is difficult.[210] In general, all the 5-$HT_3$ receptor antagonists share a number of characteristics, including a wide therapeutic margin, a threshold effect with little or no dose response, minimal toxicity, and high cost.[195,206,208,271,284,285 and 286] Although the pediatric literature is very limited, major differences from the adult literature have not been identified. Of note, remarkably few data about the use of 5-$HT_3$ receptor antagonists in infants have been published.[287] The choice of agent to use for antiemetic prophylaxis in adults can be driven by such factors as cost and convenience. The relevance of these factors in children, however, may be limited, owing to such considerations as limited dose sizes and potential difficulty with compliance with oral regimens. Some studies have demonstrated decreased efficacy for tropisetron or dolasetron as compared to ondansetron and granisetron, but final conclusions about differences cannot be determined.[225,288,289 and 290] One randomized, double-blind pediatric study found no differences between ondansetron and granisetron in the bone marrow transplant setting.[163]

**Steroids**

Although their mechanism of action is not understood, steroids have been used as antiemetic agents. Dexamethasone is the most extensively evaluated steroid, with doses ranging from 5 to 48 mg in single and multiple doses.[291,292,293,294 and 295] Recent studies focus on doses of 10 to 20 mg per day, but administration of five daily doses totaling 120 mg per day have been reported.[244] Dexamethasone and metoclopramide have been shown to have similar efficacy in adults receiving moderately and highly emetogenic chemotherapy.[292] For adults receiving moderately emetogenic chemotherapy, ondansetron was somewhat more effective than dexamethasone.[296] Although only moderately effective alone, dexamethasone is highly effective when used to potentiate the efficacy of other antiemetics.[294] The addition of dexamethasone significantly improves emesis control in patients receiving metoclopramide and all the 5-$HT_3$ receptor antagonists.[223,280,297,298,299 and 300] One of the few studies to evaluate the dose-effect of dexamethasone in conjunction with another agent found significant improvements in complete control with dexamethasone doses of at least 10 mg and only slight additional benefit with doses of 20 mg. Several studies have also shown that dexamethasone is effective in the control of delayed vomiting and that its efficacy in this setting is increased when used in combination with metoclopramide but not with 5-$HT_3$ receptor antagonists.[201,221,301] Overall, dexamethasone appears to be a safe, effective adjunct to antiemetic regimens.

The risks and benefits of dexamethasone must be considered carefully in certain clinical and research scenarios. Dexamethasone may adversely affect the efficacy of a biologic response modifier by increasing immunosuppression. Sudden withdrawal of a steroid may exacerbate radiation pneumonitis in patients who have received prior lung irradiation.[302] Dexamethasone may have a direct antitumor effect in lymphoid malignancies and so may affect the interpretation of research studies. No clear dexamethasone dose guidelines are available for children. Our starting dosage is 10 mg/$m^2$ to a maximum dose of 10 mg, given once daily. In the most symptomatic patients, the total daily dosage is doubled, and divided into a twice daily regimen with a maximum of 10 mg given twice daily. Early studies with methylprednisolone have not led to more recent work.[303]

**Phenothiazines**

Prior to the availability of 5-$HT_3$ receptor antagonists, the phenothiazines and metoclopramide were the mainstay of therapy for children. At the usual therapeutic doses, many phenothiazines appear to depress CTZ activity and also may directly depress the vomiting center.[165] Two distinct chemical classes of phenothiazines exist, each with its own therapeutic and toxic characteristics. The aliphatic class, of which chlorpromazine (Thorazine) is the prototype, has limited antiemetic activity and is associated with a high incidence of orthostatic hypotension, sedation, prolongation of the sedative effects of narcotics and barbiturates, and blood dyscrasias. The piperazine class, which includes prochlorperazine (Compazine), thiethylperazine (Torecan), and perphenazine (Trilafon), has pronounced antiemetic activity but is associated with an increased incidence of extrapyramidal effects. Evidence in dogs indicates that the efficacy of perphenazine at nontoxic doses is 24 times greater than that of chlorpromazine and 8 times greater than prochlorperazine in preventing apomorphine-induced emesis.[304] The major disadvantages of these agents—the development of extrapyramidal reactions or agitation—can be decreased by very slow (45 to 60 minutes) intravenous administration with concomitant administration of an antihistamine such as diphenhydramine (Benadryl). Although generally immediate, the side effects can appear as much as 48 hours after drug administration. Thus, repeated dosing of diphenhydramine for an additional 24 hours is recommended for patients who receive prolonged courses of phenothiazines.

The doses and routes of administration vary among the phenothiazines. Thiethylperazine, available for intravenous, intramuscular, oral, and rectal administration, should be given in 10-mg doses every 6 to 8 hours for children aged 12 years or older and in 5-mg doses for younger children. Use of thiethylperazine is not recommended for children younger than 2 years. The recommended loading dose of perphenazine is 2 to 5 mg intravenously over 60 minutes, depending on the age of the child. The loading dose can be followed by either a continuous infusion of 0.25 to 0.5 mg per hour or an oral dose of 2 to 4 mg every 4 hours. Prochlorperazine is considered to be the safest phenothiazine in children younger than 5 years but has only minimal antiemetic efficacy. The development and widespread use of the serotonin receptor antagonists led to the near abandonment of the phenothiazines. More recent studies suggest that phenothiazines can be used in conjunction with the serotonin receptor antagonists to achieve better control of symptoms.[305,306]

**Metoclopramide**

Metoclopramide (Reglan), a procainamide derivative, has both central and peripheral antiemetic actions: It inhibits chemotherapy-induced vomiting and accelerates gastric emptying. Because of its short half-life, it must be administered frequently.[307,308 and 309] The standard regimen in adults has been 2 mg per kg 30 minutes before chemotherapy and again at 1.5, 3.5, 5.5, and (sometimes) 8.5 hours after chemotherapy. Anecdotal evidence and our experience suggest that a dose of 1 mg per kg intravenously over 60 minutes repeated every 2 to 4 hours for a total of five doses is effective. Children are at higher risk for extrapyramidal symptoms than are adults, and so prophylaxis with diphenhydramine is required.[310] The incidence of akathisia and extrapyramidal reactions increases greatly at doses in excess of 1.5 mg per kg.[310] Note that much lower doses of metoclopramide (e.g., 0.2 mg per kg every 6 hours) are effective for postoperative nausea or for acceleration of gastric emptying.

**Miscellaneous Agents**

Although not a true antiemetic, *lorazepam* has proved particularly useful in combination.[311,312,313 and 314] Lorazepam is useful both for its amnestic and its anxiolytic effects. It produces anterograde amnesia and therefore is useful in allaying anticipatory nausea and vomiting by causing patients to forget their previous experiences with chemotherapy.[315] Lorazepam should always be used in combination with a true antiemetic. The dosage of lorazepam is 0.025 to 0.050 mg per kg intravenously or orally, administered 30 minutes before chemotherapy and repeated every 6 hours as needed.[313,315] To avoid perception disturbances associated with higher doses, it is prudent to start with a lowest appropriate dose and avoid exceeding 1 mg.

*Scopolamine* (hyoscine) is a potent anticholinergic agent available in a transdermal patch for the treatment of motion sickness. It has been reported to be effective as a single agent in preventing emesis due to methotrexate and in combination to prevent cisplatin-induced emesis.[316] Its major side effects are sedation and dry mouth.

The transdermal patch should be applied the evening before or the morning of intended chemotherapy. Because of the fixed dosing, it cannot be used in small children.

The *cannabinoids*, the active ingredients of marijuana, have proven antiemetic properties. Delta-9-tetrahydrocannabinol (THC) has been shown to be more effective than both placebo and prochlorperazine in preventing vomiting in patients receiving antineoplastic drugs. [317,318 and 319] Although the exact mechanism of action is unknown, recent work suggests that THC acts as a ligand for a newly-described class of specific cannabinoid receptors, both in the periphery and in the central nervous system. Many patients report relief of nausea while reporting no sedation. Interest in the cannabinoids has led to the development of synthetic analogs of THC, one of which is commercially available as dronabinol (Marinol). Although cannabinoids are not considered first-line therapy, they have been effective in children, in dosages ranging from 2.5 to 7.5 mg per $m^2$.[320] Side effects range from drowsiness to dysphoria, the most common side effect being the development of a "high."[318]

Although they have no direct antiemetic activity, *barbiturates* such as pentobarbital can be used for their sedative effects when patients experience breakthrough vomiting. Although a theoretical risk of aspiration exists in patients with a depressed level of consciousness, the emetic impulse is such a powerful stimulant that patients usually awaken to vomit and then return to their somnolent state. Given the potential risk, however, this approach is recommended only in a controlled setting when all other therapeutic avenues have been exhausted.

*Droperidol* (Inapsine), a butyrophenone, is a potent inhibitor of the CTZ. In numerous studies, using doses ranging from 0.5 to 2.5 mg as single or multiple doses or by continuous infusion, droperidol has been found to be somewhat effective.[321] Reported toxicities include hypotension, tachycardia, somnolence, agitation, and extrapyramidal effects. Experience with droperidol in young children is very limited. In older children, a loading dose of 2.5 mg intravenously over 60 minutes followed by a continuous infusion of 1 mg per hour is recommended.

Many *antihistamines* have mild antiemetic properties,[322] but no correlation can be drawn between antihistaminic potency and antiemetic activity. Both dimenhydrinate (Dramamine) and diphenhydramine are effective antiemetics for motion sickness and may be used in combination with other antiemetic therapy to potentiate effectiveness or to decrease toxicity.

### *Pharmacologic Approaches to Control of Delayed Symptoms*

The control of delayed symptoms has received far less attention in the literature than the control of acute symptoms. Initial studies demonstrated the marked efficacy of the combination of dexamethasone and metoclopramide after the administration of high-dose cisplatin.[201] Dexamethasone alone is effective for patients receiving moderately emetogenic regimens.[301] A recent study has shown significant efficacy in the control of delayed symptoms with a three-drug combination of granisetron on day 1, dexamethasone on days 1 through 5, and prochlorperazine on days 1 through 3.[323] Another encouraging study piloted the use of delayed symptom treatment beginning at hour 16 after cisplatin infusion.[324] The role of 5-$HT_3$ receptor antagonists in the management of delayed symptoms is less clear. Although several studies have shown a benefit to a 5-$HT_3$ receptor antagonist as compared to placebo, other studies have shown no benefit to a 5-$HT_3$ receptor antagonist as compared to dexamethasone alone or metoclopramide alone.[195,220,221,224] Clear evidence of increased incidence of constipation in patients receiving prolonged courses of 5-$HT_3$ receptor antagonists also exists.[220,221] The 5-$HT_3$ receptor antagonists may have increased utility for patients with poor control of acute symptoms. Until more clear-cut studies are conducted that demonstrate the utility of the 5-$HT_3$ receptor antagonists in the control of delayed symptoms, cost should preclude routine use of these agents. Further studies are needed to determine the role of substance P inhibitors in the control of delayed symptoms.[183,184,186,325]

### Approach to Therapy

Clinical practice guidelines developed in the adult setting have focused on the appropriate use of prophylactic regimens both to improve symptom control and to decrease costs.[210,218,227] As in adults, any such guidelines for children must address the acute and late emetogenicity of the regimen and the efficacy and side effects of the agents available. In addition, however, pediatric guidelines must also consider limited dose forms and how best to extrapolate from the adult literature.[326] Table 43-7 summarizes the clinical practice guideline in use at our institution regarding agents to be used with the first administration of any chemotherapeutic regimen. It is critical to adjust the prophylactic regimen used in subsequent courses to the pattern of symptoms and response to prophylactic and any rescue agents used in the prior course of the same agents. In general, we recommend a 5-$HT_3$ receptor antagonist alone for mildly and moderately emetogenic regimens and in combination with dexamethasone for more emetogenic regimens as initial therapy for all patients. Although there is a lack of published experience with ondansetron in very young children, we and others have used ondansetron in infants. For prevention of delayed emesis, we use dexamethasone with or without metoclopramide (with diphenhydramine to prevent extrapyramidal symptoms). For breakthrough emesis, we recommend the sequential addition of dexamethasone, lorazepam, scopolamine (if weight is more than 40 kg), dronabinol (if older than 6 years) or metoclopramide (with diphenhydramine). Because pentobarbital is not currently available, we no longer use a soporific agent. If more than one or two doses of an agent that can cause extrapyramidal symptoms are administered on one day, we recommend continuing diphenhydramine for an additonal 24 hours. Because anticipatory and psychogenic nausea and vomiting can be a significant problem, the most effective regimen is one that prevents nausea and vomiting in the chemotherapy-naive patient. Success or failure of a particular regimen is ultimately determined by the patient's acceptance of future courses of chemotherapy. Although complete elimination of symptoms may not be possible in all patients, a substantial reduction in both the degree and duration of symptoms can usually be achieved, making the treatment more tolerable.

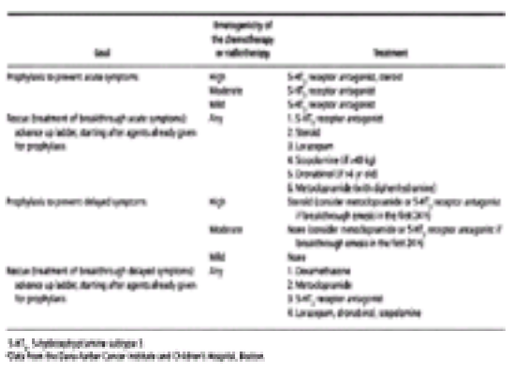

**TABLE 43-7. ANTIEMETIC ALGORITHM FOR INITIAL CYCLE OF CHEMOTHERAPY OR RADIOTHERAPY[a]**

### Somnolence, Fatigue, and Insomnia

Sleep disorders, daytime sedation, fatigue, and lack of mental clarity are common problems among children undergoing cancer treatment, in the setting of advanced cancer, and in some long-term cancer survivors. Fatigue or lack of energy is extremely common among children with cancer.[13,327,328 and 329] These symptoms can occur during treatment with chemotherapy or radiation, after surgery, in the setting of advanced disease, or even in long-term in disease-free survivors.[320] The causes of fatigue often are multifactorial and may reflect a combination of medical and psychosocial factors.[331,332] Sleep disturbance, depression, and anxiety often are associated with daytime fatigue. Depressed mood and anxiety are common in patients with cancer; in most cases, these subjects had no history of mood disorders prior to the onset of the cancer.

One's approach to the child with fatigue should include identification of remediable medical factors (e.g., anemia, cachexia, endocrine or electrolyte disturbances). When depression or anxiety is present, consideration should be given to psychotherapy and pharmacologic treatment. Fatigue often coexists with sleep disturbance. Lifestyle change, including improved sleep hygiene and avoidance of caffeine in the evening, may be helpful to improve sleep. Low doses of an antidepressant are our preferred pharmacologic treatment of persistent sleep disturbance. Fatigue and somnolence, especially in patients taking opioids, may improve with administration of methylphenidate or other stimulants.[149] Graded aerobic exercise programs have shown promise[333]; they may also improve sleep and ameliorate depressed mood.

### Constipation

Constipation is common among patients with cancer. Often but not exclusively, it is due to opioids. [334] Some studies suggest differences among opioids in the severity of constipation. For example, in some studies, transdermal fentanyl was found to produce less constipation than oral morphine. [335]

Although laxatives often are prescribed, they frequently are ineffective, for several reasons: (a) They often are not administered even when prescribed, (b) they often are administered in fixed doses, even when ineffective, and (c) they often are administered only after constipation is persistent and severe. [336]

### Dyspnea

Dyspnea is a common and distressing symptom in pediatric palliative care. [119] Among adults, it is a common feature of lung and head and neck malignancies. [337] In children, dyspnea is commonly seen with solid tumors metastatic to the lungs and pleural spaces. The causes of dyspnea are multifactorial and may be associated with obstructive, restrictive, or mixed abnormalities on spirometry, as well as hypoxemia or hypercarbia. Dyspnea may also result from extrapulmonary causes, including generalized cachexia and fatigue that affects the respiratory as well as other muscles. [338]

In selected cases, palliative interventions may include palliative chemotherapy or radiotherapy, diuretics, pleurocentesis with or without chest tube drainage, pleurodesis, and supplemental oxygen. Occasional patients with reactive bronchoconstriction respond to bronchodilators.

Opioids often are beneficial in reducing the distress of dyspnea, whether due to increased work of breathing or due to hypoxemia or hypercarbia. In the majority of cases, opioids improve dyspnea without inciting significant respiratory depression. [339] Although dyspnea often is accompanied by anxiety, and benzodiazepines frequently are administered to patients with dyspnea, controlled trials have generally found benzodiazepines ineffective in the treatment of cancer-associated dyspnea. [340]

Some palliative care centers use inhaled morphine specifically for dyspnea, although the evidence regarding whether this agent works by a local versus a systemic mechanism remains controversial. Opioid receptors are present at comparatively high density in many parts of the tracheobronchial tree, particularly at the level of bronchioles and lung parenchyma. [341]

## CONCLUSION

Management of symptoms and suffering is an essential part of the care of children with cancer. Each symptom should not be viewed in isolation but rather in the context of the individual child's overall situation. Despite the limitations and imperfections of available methods of treatment, attentive care and the application of pharmacologic principles should allow physicians to ameliorate their patients' distress in the great majority of circumstances. More prospective research is needed on the clinical pharmacology of medications used for symptom management in children and on outcomes of different treatment approaches.

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# 44

# NURSING SUPPORT OF THE CHILD WITH CANCER

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## INTRODUCTION

Pediatric oncology nurses are essential contributors to the successful treatment and cure of children with cancer. As a member of the multidisciplinary care team, the nurse works with physicians, social workers, child life specialists, school teachers, psychologists, chaplains, and other specialists to provide comprehensive care for the child and family. Innovative technologies require that nurses caring for children with cancer become experts in critical care management as well as in the provision of psychological support to the child and family. The expert oncology nurse often functions as the coordinator of patient care, facilitating communication among team members.

Pediatric oncology nursing roles are diverse and allow for opportunities in direct patient care, education, management, and research. Advanced practice nurses have made significant contributions toward improved nursing care for children with cancer. Children are followed in various patient care settings, including the hospital, outpatient setting, and home environment. Nurses in all these care settings play a unique role in the management of childhood cancer.

## DIRECT PATIENT CARE NURSING ROLES

Nurses caring for the child with cancer in the hospital setting must keep pace with the complex advances in treatment as well as with advanced technology. The nature of acute care settings may change even more dramatically in the future. As the trend in pediatric oncology continues with more therapies being administered in the clinic setting, inpatient services will be largely used for the management of acute life-threatening complications or for the administration of therapies that cannot be administered in the ambulatory setting. Future inpatient care settings will consist of multiple intensive care units offering highly specialized scientific and technologic services.[1]

The nurse working with children in these tertiary care settings must be able to recognize short-term as well as long-term physical needs. [2] Expert nursing care requires the ability to assess the child's condition based on extensive knowledge of childhood cancer, to develop a plan of care in collaboration with other health care professionals, to provide direct nursing care for the child, and to evaluate the child's condition based on specific nursing outcomes. Nurses caring for children with cancer must competently manage symptoms of common side effects of treatment such as nausea and vomiting, pain, mucositis, and anorexia. Hospital nurses are integral for providing education and support to families with a child newly diagnosed with cancer. Crisis intervention and the ability to provide emotional support are essential. The nurse also frequently serves as a child advocate, ensuring proper preparation for invasive procedures and treatment. Because many patients receive much of their treatment in the hospital, nurses develop long-term relationships, providing continuity of care for these families.

The nurse working in the clinic setting is often the direct link between the community and the cancer treatment center. Pediatricians who follow cases of children with cancer in community settings may communicate directly with the nursing staff regarding specific side effects or laboratory findings. Clinic nurses frequently provide education regarding administration of chemotherapy in the pediatrician's office. Extensive knowledge of the side effects of treatment enables the nurse to provide families with an understanding of what may occur at home. Clinic nurses also communicate information regarding specific restrictions and changes in activities for the child to the family.

As experts in the administration of complex chemotherapy regimens, clinic nurses enable children to remain out of the hospital while undergoing treatment. Children are now receiving in the clinic setting chemotherapy that could previously be administered only in a hospital. The use of sedation before invasive procedures is common practice in many cancer centers. Clinic nurses have extensive knowledge regarding assessment and management of children receiving sedation. As a consequence of intensive chemotherapy regimens, blood product support is also frequently necessary and is administered in the outpatient setting by nurses who are knowledgeable regarding possible reactions and who expertly manage side effects related to blood product transfusion.

Families who have children with cancer return to the outpatient setting frequently for treatment and follow-up. The nurse becomes a major support to the child and family throughout treatment. Once therapy is completed, families continue to use the nurse as a major resource for their questions and concerns.

## ADMINISTRATIVE ROLES

Nurses in administrative roles face the challenge of implementing cost-effective, high-quality care to increasingly ill patients who have complex health care needs. These nurses must have an extensive background in nursing as well as in business. Managed care is changing health care, and nurses in administration will be instrumental in coordinating care in accordance with specific health care plans in the future. Administrative nurses must support the specialization of oncology nursing while meeting the demands of changing health care systems. A major concern is to direct efforts toward recruitment and retention of professional, skilled pediatric oncology nurses who will deliver high-quality care to children and their families.

## ADVANCED PRACTICE ROLES

One of the most significant contributions of nursing has been the development of advanced practice roles. [2] Such nurses are experts in the clinical care of children with cancer. They serve as the coordinators of care among hospital, clinic, and community settings. The nurse practitioner must understand the assessment and management of children with cancer. Proficient in performing physical assessments, completing diagnostic procedures such as bone marrow aspirations and lumbar punctures, and diagnosing common pediatric illnesses, the nurse practitioner has developed a major role in the outpatient setting.

Since the conception of their role, nurse practitioners have demonstrated ability to provide appropriate, cost-effective care for a range of health services, including primary care, management of chronic illness, and treatment of episodic health problems. In recent years, positions for nurse practitioners have been created in specialty areas, such as oncology, as physicians and health care administrators have recognized the quality and cost-effectiveness of the role. [3]

Clinical nurse specialists use their expertise by helping other team members to coordinate care, usually during the patient's hospitalization. The clinical nurse specialist complements the role of the nurse practitioner by providing continuity between the clinic and the hospital. Whereas the nurse practitioner directly cares for a

selected population of patients, the clinical nurse specialist often serves as a coordinator of care for all children who are hospitalized. Communication between staff nurses and the clinical nurse specialist is key in providing information from the health care team managing the child's care. The clinical nurse specialist is instrumental in implementing organized teaching programs for parents and children. Both the clinical nurse specialist and the nurse practitioner serve as resources for other nurses.

Advanced clinical practice in pediatric oncology nursing requires preparation at the master's degree level. Graduate programs are designed to prepare advanced practice nurses to think independently, function autonomously, and participate actively within an interdisciplinary team. [4,5] An extensive knowledge base in physiology, child health assessment, growth and development, health promotion, disease prevention, and management of common problems of childhood is essential. Once a foundation of knowledge regarding well-child care is established, graduate nursing programs should provide opportunities for experiences in the care of the child with cancer. Didactic content in the pathophysiology, diagnosis, and management of the various types of childhood cancer is essential, yet general pediatric graduate programs often do not include these topics in the curriculum. Nurses seeking to specialize in pediatric oncology should pursue opportunities to care for children with cancer during graduate nursing study.

Many advanced practice nurses who join comprehensive childhood cancer centers have limited knowledge of the diagnosis, treatment, and management of cancer. Cancer centers must consider developing innovative educational opportunities that provide the knowledge necessary to pursue advanced practice roles. Short-term fellowship programs that allow for clinical participation under the supervision of experienced nurses may become an important investment as nurses' functions become more independent in the future.[6]

## RESEARCH ROLES

Nurses with diverse educational backgrounds and experience are also involved in research roles. Educational preparation influences the types of research roles nurses pursue. Baccalaureate-level nurses typically participate in research by evaluating its applicability for nursing practice. They assist in the identification of research problems and are involved in research implementation by serving as data collectors and by obtaining subjects for study.

Numerous cancer centers also use baccalaureate-prepared research nurses as coordinators of clinical trials. The research nurse ensures that the study is implemented according to protocol and that data collection is accurate.[7] Phase I clinical trials are excellent examples of studies coordinated by research nurses; the research nurse implements labor-intensive regimens according to protocol and closely monitors side effects and toxicity data.

The nurse with a master's degree has the expertise to identify practical problems for clinical relevance and to facilitate implementation of nursing research. The nurse in advanced practice enhances the value of research among other nurses by participating in collaborative research endeavors. Doctorally prepared nurses are increasing in number and serve as nursing research directors at numerous institutions. They promote interest in research and are instrumental in implementing funded nursing research projects.

## NURSING STANDARDS OF CARE

The image of pediatric oncology nursing is reflected in the standards of care practiced daily by nurses. The outcome standards of pediatric oncology nursing practice, established by the Association of Pediatric Oncology Nurses, reflect the comprehensive involvement of nursing in the care of children with cancer.[8] These standards assist in identifying the future focus of pediatric oncology nursing care and include providing expert clinical care, coordinating patient and family education, facilitating psychosocial support, promoting growth and development, following up long-term survivors, and continuing professional development. These outcome standards are the framework for nursing care discussed in this chapter.

### Providing Expert Clinical Nursing Care

Nurses caring for children with cancer must keep pace with complex advances in treatment and technology. Expert nurses are able to assess the child's condition using extensive knowledge of childhood cancer, to develop a plan of care in collaboration with other health care professionals, to provide direct nursing care, and to evaluate the child's condition based on specific nursing outcomes. Pediatric oncology nurses play a major role in managing disease- and treatment-related side effects, coordinating care for central venous lines, administering chemotherapy, and preparing the child for invasive procedures. As more children are treated in the outpatient or home environment, nurses have become the coordinators of care in these settings.

### Managing Side Effects

The management of treatment-related side effects is a routine aspect of the nurse's role. Frequent problems include infection, bleeding, anemia, nutritional concerns, nausea and vomiting, mucositis and pain.

#### *Myelosuppression and Consequent Infection*

Chemotherapy agents and radiation therapy cause myelosuppression. In addition, certain malignancies involving the bone marrow (e.g., leukemia, lymphoma, and neuroblastoma) cause a decrease in the number of normal blood cell precursors. When the effect is severe enough, the child becomes predisposed to infection, anemia, or bleeding, depending on which cell line is affected. Infection resulting from neutropenia may be life threatening (see Chapter 41). After cytotoxic chemotherapy, or during long courses of radiotherapy, the bone marrow cannot produce an adequate number of neutrophils to protect against infection. A patient with an absolute neutrophil count of 500 $mm^3$ is considered neutropenic. Children who have prolonged periods of neutropenia (i.e., 7 or more days) are considered high-risk patients and may develop one or more secondary infections. The neutropenic child will not demonstrate the normal signs and symptoms of infection. Fever may be the only indication that infection is present.

The nurse plays an important role in minimizing the risk of infection in these children. Most infections in the neutropenic child are caused by endogenous flora; however, adequate protection from infection is the best defense. Hand washing before and after contact with each patient minimizes the risk of microbial transmission and is the single most important method of preventing nosocomial infection.[9] Administration of biologic response modifiers (e.g., granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor) has decreased the duration of neutropenia after cytotoxic chemotherapy. The use of these agents for patients with a short period of neutropenia is generally not indicated (see Chapter 10).[10,11]

Certain pathogens that are particularly dangerous to immunosuppressed children are listed in Table 44-1. When a neutropenic child develops fever, blood cultures from both central [e.g., implanted central venous access device (CVAD)] and peripheral sources are obtained, as well as cultures of other appropriate body fluids or sites (e.g., throat, urine, wound, lesions, and catheter exit site). Broad-spectrum intravenous antibiotics are initiated. Antibiotic therapy is modified based on the culture and sensitivity of the organisms isolated.

| |
|---|
| Viruses |
| Varicella-zoster |
| Herpes simplex |
| Epstein-Barr |
| Cytomegalovirus |
| Adenovirus |
| Measles |
| Respiratory viruses |
| Gram-negative bacteria |
| *Escherichia coli* |
| *Pseudomonas aeruginosa* |
| *Klebsiella pneumoniae* |
| *Enterobacter sp* |
| Gram-positive bacteria |
| Coagulase-negative staphylococci |
| *Staphylococcus aureus* |
| *Streptococcus pneumoniae* |
| α-Hemolytic streptococci |
| Group A β-hemolytic streptococci |
| Enterococcus |
| Protozoa |
| *Toxoplasma gondii* |
| *Pneumocystis carinii* |
| Fungi |
| *Candida sp* |
| *Aspergillus sp* |
| *Mucormycosis* |

**TABLE 44-1. COMMON PATHOGENS THAT CAUSE INFECTION IN IMMUNOCOMPROMISED CHILDREN**

Nursing care of the child hospitalized with fever and neutropenia is directed toward monitoring for signs of septic shock. Vital signs must be monitored at least every 4 hours for fluctuations in temperature (very low or very high), heart rate, respiratory rate, blood pressure, and urine output. Because hypotension is usually a late sign of shock in children, peripheral perfusion should be checked frequently. Delayed capillary refill and tachypnea are early signs of impending shock. The child's level of consciousness must be assessed continually for irritability, lethargy, or unresponsiveness. Temperature measurement by the rectal route and the use of suppositories and enemas must be avoided in neutropenic children. Mouth care and perianal hygiene must be done on a regular schedule. If the child is febrile (temperature greater than 38.5°C), cultures are obtained, and acetaminophen administered. The parents should be given an opportunity to ask questions during the period of acute serious illness (e.g., fever and neutropenia and septic shock), as this time is often confusing and stressful for the family.

Parents and children must be educated regarding the prevention of infection. All members of the family must practice strict hand washing to decrease the spread of pathogens among each other. The parents must know when the period of neutropenia is likely to occur after chemotherapy. If fever is suspected, they should take the child's temperature by the oral or axillary route, but never rectally. If the child's temperature is 38°C on three or more occasions in a 24-hour period, or 38.5°C or higher, the parents should notify the nurse or physician immediately and should not administer acetaminophen unless instructed to do so.

Adequate nutrition is an important component in the prevention of infection. Cancer treatments often cause anorexia, nausea, and vomiting, which make adequate dietary intake difficult to achieve. Food should never be forced on the child, and should alternate feeding plans be required (e.g., gastric tube feedings or total parenteral nutrition), care must be taken to use the appropriate sterile technique to prevent infection.

Varicella-zoster virus infection can present a potentially life-threatening danger to the child undergoing cancer therapy if the child has never had chickenpox (see Chapter 41). Parents must be aware if their child is exposed to chickenpox, and school nurses, teachers, neighbors, and parents of playmates must be educated regarding this danger. Parents must be notified when playmates, classmates, or other contacts develop chickenpox. Infected persons are contagious for 1 to 2 days before the onset of the vesicular rash and throughout the duration of eruption. If the immunocompromised child is directly exposed to an infected person, varicella-zoster immune globulin (125 U per 10 kg intramuscularly, maximum dose, 625 U) should be administered within 96 hours. [9] Direct exposure is defined as having an infected household contact, 1 hour or more of indoor play with an infected person, or hospital exposure through prolonged face-to-face contact with an infected health care worker or patient. The exact duration of effectiveness of varicella-zoster immune globulin is unknown. If another exposure occurs longer than 3 weeks after the injection, the dose is administered again. Hospitalized children with varicella-zoster virus must be placed in strict isolation for up to 28 days after exposure. Immunocompromised children who develop varicella infection may be treated with intravenous acyclovir alone, [9] although some children with mild or uncomplicated cases may receive intravenous (i.v.) acyclovir followed by oral acyclovir and reduce the duration of i.v. treatment and hospitalization. [12] Health care workers who have not had chickenpox should be advised to receive the varicella vaccine. [13,14] After an individual has had chickenpox, varicella-zoster virus persists in a latent form. Immunosuppression from chemotherapy or radiation can reactivate the virus. Vesicular lesions appear along a sensory dermatome. This is known as *zoster* or *shingles*. The eruption of lesions is preceded by a prodrome of pain or tingling. Some patients may not manifest the typical vesicular cutaneous changes and may only describe sensations of pain or tingling, which may or may not lie within a dermatome. Treatment of patients that have zoster is similar to that of patients with primary varicella infection.[9]

Nursing care of the child with varicella infection requires strict attention to good hygiene and hydration, fever control, and management of pruritus. These children must be continually assessed for evidence of disseminated infection or secondary bacterial infection. Ocular involvement, pneumonia, hepatitis, meningitis, and encephalitis (i.e., progressive disseminated varicella) may occur. [15,16]

*Pneumocystis carinii* is generally not pathogenic in a healthy host, but it can cause a life-threatening pneumonia in persons who are immunosuppressed. This condition is almost entirely preventable. Trimethoprim-sulfamethoxazole, 150 mg per $m^2$ of the trimethoprim component orally divided into two doses, given 3 consecutive days each week, is adequate prophylaxis. For patients who are unable to take trimethoprim-sulfamethoxazole because of hypersensitivity reaction or bone marrow suppression, dapsone, 2 mg per kg (maximum 100 mg per day) orally once daily, is also effective. Aerosolized pentamidine, 300 mg per dose, is another option for *P. carinii* pneumonia prophylaxis and is administered once monthly.[17] The patients must be old enough (usually 5 years or older) to cooperate with aerosolized drug administration via the Respirgard II inhaler and must come into the clinic to receive the medication. Although pentamidine has been shown to prevent *P. carinii* pneumonia, its administration is labor intensive, and it is certainly more costly than the medications that can be administered at home.

Respiratory syncytial virus and cytomegalovirus are other potentially problematic infections for children with cancer, especially those undergoing bone marrow transplantation. Other respiratory viruses, including adenovirus and influenza, generally do not cause more severe disease in children with cancer than in more immunocompetent patients.

### *Administration of Immunizations*

Live virus vaccines are contraindicated in children receiving immunosuppressive therapy because of potentially serious adverse effects. Vaccine-strain poliomyelitis, measles virus, and vaccinia have been reported in immunocompromised children after administration of live virus vaccines. [9] Immunologically normal household contacts of immunocompromised children should receive inactivated poliovirus vaccine, because live poliovirus is transmissible after immunization with oral poliovirus vaccine. Live measles, mumps, and rubella vaccine can be administered to the siblings and household contacts of children with cancer because these viruses are not transmissible after vaccination. Varicella vaccine has been given to nonimmune household contacts of children with cancer without transmission of the virus to the immunosuppressed child.[18] Therefore, it is recommended for susceptible contacts of these children. [9,19] Children who have received chemotherapy or radiation therapy should not be given live virus vaccines until at least 6 months after immunosuppressive treatments have ceased. The degree of immunosuppression and its duration may vary among patients, however.[19] Other routine childhood immunizations, such as diphtheria-tetanus-pertussis, *Haemophilus influenzae* type b conjugate, and hepatitis B, can be administered safely on a standard schedule, although immunogenicity may be reduced. Children aged 24 months or older who have Hodgkin's disease should receive pneumococcal and meningococcal vaccines, because these children are at increased risk of infection from these organisms. [9,19]

### *Bleeding and Anemia*

Children with cancer are at risk of developing bleeding related to thrombocytopenia or coagulopathy. Anemia may occur due to blood loss or a decrease in the production of red blood cells related to bone marrow suppression from cancer treatment (see Chapter 40). Children who are at risk for bleeding (platelet count less than 100,000 $mm^3$) should be placed on precautions, so the potential for bleeding can be decreased. However, spontaneous internal hemorrhage does not occur until the platelet count is 20,000 per $mm^3$.[20,21] and [22] Nurses should educate the family and child to avoid ibuprofen, aspirin, and aspirin-containing products. Minor pain, and fever without neutropenia, are treated with acetaminophen. The child's body temperature should not be taken rectally. The use of razors should be avoided, and a soft toothbrush should be used for dental care. Children should avoid using dental floss to prevent traumatic injury to gingival tissue. Adolescent female patients may be given oral contraceptives or hormone therapy to suppress menses to decrease the risk of excessive bleeding. Eating or chewing sharp foods (e.g., corn or tortilla chips and ice) should be avoided to prevent gingival bleeding. Contact sports or activities that may cause injury or bleeding (e.g., football, soccer, bicycling, skateboarding, and tree climbing) should not be permitted. Venipunctures and other invasive procedures (e.g., lumbar puncture and bone marrow aspiration) should be performed with caution when platelet counts are low.

If the child experiences epistaxis, the parents should be instructed to pinch the child's nostrils together with a gauze pad held between the thumb and index finger for at least 10 minutes. If there is persistent bleeding, or if the patient experiences hematuria or hematochezia, the child should be evaluated at the hospital. If the child is admitted to the hospital with thrombocytopenia, nursing interventions include measures to prevent injury, inspection of body fluids for evidence of blood, monitoring of vital signs and peripheral perfusion for evidence of blood loss, and administration of platelet transfusions.

Children may become anemic from blood loss or as a consequence of chemotherapy-induced myelosuppression. Children are amazingly resilient and tolerate low hemoglobin concentrations well. Signs and symptoms of anemia include pallor, headache, dizziness, shortness of breath, fatigue, tachycardia, and heart murmur. Packed red blood cell transfusion is generally required when the hemoglobin falls below 7 g per dL.

When red blood cell transfusions are required, leukocyte-depleted or irradiated blood products are often administered. Lymphocyte reduction of packed red blood cells and platelets is used to prevent HLA-alloimmunization and refractoriness to allogeneic platelet transfusion, nonhemolytic transfusion febrile reactions, and graft-versus-host disease.[23,24,25,26] and [27] Irradiation of cellular blood components is used to prevent posttransfusion graft-versus-host disease. [25,26,28,29] and [30] The decision to administer lymphocyte-depleted or irradiated blood products depends on the child's immunologic status and on the intensity of the chemotherapy regimen.

All children who are bone marrow transplant recipients should receive leukocyte-depleted, irradiated blood products.

Transfusion of blood and platelets may cause transfusion reactions, manifested by fever, chills, body aches, urticaria, pruritus, and, in severe cases, wheezing and respiratory compromise. The transfusion should be discontinued and intravenous normal saline infused. Antihistamines or steroids may be administered. [27] Parents may prefer to limit blood and platelet transfusions to designated donor products if the situation is not an emergency or if the child is not a potential bone marrow transplant recipient. The use of designated donor blood products may reduce the incidence of transfusion reaction if a parent or sibling's blood products are compatible. The hospital blood bank can provide information and instructions regarding specific designated donor programs.

### *Nutritional Changes*

Alterations in nutritional status in the child undergoing cancer treatment are common (see Chapter 42). The disease itself and the side effects of therapy (e.g., nausea, vomiting, anorexia, stomatitis, dysphagia, and changes in taste) often interfere with adequate caloric intake. Conversely, the use of glucocorticoids (i.e., prednisone and dexamethasone) causes an increased appetite and an intense craving for salty foods. When these drugs are given, weight gain may be excessive. In either case, the patient's weight should be checked at each visit and plotted at regular intervals on a growth curve.

When metabolic needs exceed caloric intake, the child may benefit from a nutritional supplement given between meals. Methods to increase caloric intake include providing high-protein snacks or high-calorie ingredients in recipes. Small, frequent meals may be more appetizing if the child is experiencing nausea. If the child continues to lose weight, or drops off the growth curve, a dietitian should be consulted. The child may require total parenteral nutrition or placement of a feeding tube to prevent malnourishment (see Chapter 42).[31,32]

### *Nausea and Vomiting*

Cancer chemotherapy agents are emetogenic, and nausea and vomiting can severely alter fluid balance in the pediatric patient (see Chapter 43). Even when chemotherapy administration is preceded by antiemetic therapy, nausea and vomiting may still occur. Some patients receiving cisplatin or carboplatin experience delayed nausea and vomiting several days after the drugs are administered. While chemotherapy agents or i.v. hydration is infusing, the nurse must monitor intake and output closely and note any discrepancy that would indicate dehydration or overhydration. Patients receiving radiation therapy to the chest, abdomen, pelvis, or craniospinal axis may experience nausea, vomiting, anorexia, and diarrhea. Antiemetic or antispasmodic therapy may be indicated for these patients to provide symptomatic relief (see Chapter 43). Certain patients who experience anticipatory or treatment-associated nausea and vomiting may benefit from relaxation techniques or guided imagery. Nurses can educate the patient and family regarding these nonpharmacologic methods.

### *Mucositis*

Gastrointestinal cell damage from chemotherapy or radiation can cause ulcerations in the mucosal surface of the alimentary canal. This side effect is extremely painful. Ulcers occurring in the oral cavity are referred to as *stomatitis* and appear as edematous, erythematous, eroded lesions. These lesions may extend down into the esophagus. Anorexia commonly occurs, because eating and drinking cause extreme pain.

It is important for the child to be examined by a dentist before receiving chemotherapy likely to produce mucositis, or radiation to the head and neck. Removal of plaque and treatment of existing dental caries is essential in preventing systemic infection once myelosuppression occurs. [33] Meticulous oral hygiene assists in preventing or lessening the deleterious effects of mucositis. In infants and small children, gingival care is achieved by wrapping a gauze pad around a finger, soaking the gauze pad in saline solution, and swabbing the patient's gums, palate, and buccal mucosa. This care should be given after eating or drinking or as often as every 2 hours. Older children can cleanse their own teeth and gums with a soft toothbrush and use a saline-based oral solution to rinse the mouth. Because orthodontic appliances may harbor debris and cause infection they may need to be removed during chemotherapy. Nursing management of the child with mucositis involves implementing an oral hygiene regimen, monitoring hydration, and encouraging the child to choose foods that are best tolerated.

Prevention of infection and treatment of pain are the main objectives in treating oral mucositis. Various oral care measures, including pharmacologic management of mucositis, are summarized in Table 44-2. Daily oral care, antiseptics, topical anesthetics, coating agents, lubricants, mechanical débridement, and miscellaneous agents are used. Fluid intake can be facilitated by the use of a straw to bypass tender oral mucosa. Anorexia is expected in these children, and as the ulcerations heal they will start to eat and drink normally. Subsequent chemotherapy regimens may require dose modification to prevent similar episodes. [34]

| Category | Agents | Action | Comments |
|---|---|---|---|
| Rinses | Saline rinse (salt in warm water to taste)<br>Sodium bicarbonate rinse (1 tsp soda [baking soda] to 1 qt water) | Removes particulate matter from teeth, tongue, and gingival tissue | Safe, effective, economical |
| Antiseptics | Chlorhexidine gluconate 0.2% (Peridex)<br>Mouthrinse (viral 4.3%, glycerin 20.2%, sodium lauryl sulfate [SLS] 2%) | Antibacterial activity | Chlorhexidine may stain teeth.<br>Generally well tolerated |
| Topical anesthetics | Maalox plus diphenhydramine | Antacids reduce acidity, diphenhydramine has a mild anesthetic effect | Give 15–20 min before meals; effect brief |
| | Viscous lidocaine (Xylocaine) 2% | Anesthetic effect | Limit use if swallowing to prevent systemic toxicity. Numbness may increase danger of oral trauma or aspiration. |
| Coating agents | Orabase (with or without benzocaine) | Provides protective barrier | Colorless and tasteless. |
| | Sucralfate suspension | Cytoprotectant | Soothes inflamed tissue. |
| Lubricants | Ayre's Lube (artificial saliva) | Provides moisture | Tasteless; thick consistency. |
| Other topical agents | Vitamin E | Antioxidant | Tasteless; sticky consistency. |
| | Thalidomide (cytokine A-1 derivative) | Stimulates wound healing | Mild odor, tasteless. |
| Mechanical debridement | Soft-bristle toothbrush | Removes debris | Use gently if neutropenic or thrombocytopenic. |
| | Foam swabs (toothettes) | | Also useful for application of topical medications. |

*See text for details.

**TABLE 44-2. GUIDELINES FOR ORAL CARE***

### *Pain Management*

One of the most important roles of the nurse is the assessment and management of pain in children with cancer. Supportive care for these children involves developmentally appropriate assessment to establish effective pain interventions. Interventions designed for children experiencing pain should include nonpharmacologic strategies as well as medications when possible.

#### Common Myths about Children in Pain

Misconceptions about the child's ability to perceive pain interfere with accurate assessment and treatment of pain. Some of these myths are as follows [35,36,37,38] and [39]: (a) a child's ability to feel pain is inhibited because children have an immature nervous system; (b) a child cannot communicate the location and intensity of pain; (c) children do not remember painful events; (d) it is always possible to determine whether a child is faking pain or is truly experiencing pain; (e) pain must have an evident stimulus, and if one is not noted, then the child cannot be feeling pain; (f) a child reports pain to the nurse or doctor; and (g) if the child does not complain of pain, then the child is not in pain.

If any member of the health care team believes any of the foregoing myths regarding a child's ability to perceive pain, pain management will be inadequate. The entire assessment and intervention process will be impaired. Pain management in children has always been suboptimal, [40] and to remedy this situation, each health care professional needs to be aware of and dispel these myths.

#### Developmental Considerations

Children's perceptions of pain are influenced by the child's stage of cognitive development, cultural environment, and parent-child relationships. [41,42] These influences are important to consider when assessing the child's pain as well as when developing appropriate management strategies. Another important consideration is that the child with a chronic illness such as cancer may be more medically sophisticated than other children the same age. Children with cancer are often advanced in their knowledge of medical treatment and are acutely aware of its effects. They may not be cognitively able to comprehend the meaning of the treatment and its importance

to survival, however. This discrepancy can create conflict for the health provider caring for a 4-year-old patient who can explain the technical details of bone marrow aspiration yet is combative and out of control during the procedure.

Children develop their understanding of pain similar to Piaget's [43] conceptualization of cognitive development, although there is considerable overlap in these categories (Table 44-3). Younger children who are generally in the preoperational stage of development communicate their perceptions of pain much differently than do older children, who are capable of abstract thought. The toddler or preschool child often perceives that he or she has done something bad to cause the pain and does not understand why treatment is necessary. Children this age cannot comprehend that painful treatment is sometimes necessary to prevent the disease from recurring. For this reason, the young toddler, unable to understand the purpose of a bone marrow aspiration, should have the procedure completed as quickly and painlessly as possible. In very young children, the use of pharmacologic interventions is more appropriate and effective than trying to explain the importance of the procedure.[44]

| Age (yr) | State of cognitive development | Patient perception of pain |
|---|---|---|
| 2–7 | Preoperational | May have done something "wrong" or "bad" and is now being punished; no difference between disease-related pain and treatment-related pain. |
| 7–11 | Concrete operational | Fears pain may lead to death; past experiences influence the present situation; treatment and side effects are "worse" than the disease. |
| 11+ | Formal operational | Understands the cause of pain; realizes the difference between disease-related pain and treatment-related pain. |

**TABLE 44-3. PIAGET'S STAGES OF COGNITIVE DEVELOPMENT: INFLUENCES ON PAIN PERCEPTION IN CHILDREN**

Children aged 7 to 11 years begin to develop concrete thinking skills and are able to understand situations that infants, toddlers, and preschool children are generally unable to comprehend. During this stage, however, children have vivid imaginations and often fear that pain may lead to death. Preparation of the school-aged child before the procedure is essential to assist in alleviating unfounded fears and anxiety. Past experiences often play a major role in how the 6- to 12-year-old child perceives pain caused by cancer or required treatment. For example, a difficult procedure performed by an unskilled health care professional can cause the child to fear the procedure and to become uncooperative during future attempts. Every effort should be made to prevent experiences that unnecessarily increase the child's pain and discomfort.

Children older than 12 years generally begin to develop the capabilities for formal operational thought. [43] They are able to understand the differences between disease-related pain and the side effects of treatment. During this stage of development, adolescents may be difficult for the health care provider to interact with because these patients often express their reaction to pain by withdrawing from others or by becoming depressed. Open, honest discussions regarding the disease and its treatment may assist adolescent patients in coping with the associated discomfort. These dialogues allow for the development of effective coping skills.

The influence of the child's cultural environment should not be overlooked. Cultural beliefs can play a major role in the family's perception of cancer and its treatment. Cultural beliefs regarding pain and suffering in children should be considered when implementing intervention strategies for the child. Family assessment should include specific questions regarding the meaning of the illness and perceptions of treatment. Accurate family assessment can provide helpful information when establishing interventions for the child in pain.

Parent-child relationships directly influence the child's perception of pain, regardless of the age of the child. Parents who are distraught and are unable to control their fears and concerns often are a detriment to the child's ability to cope with the painful effects of the disease and treatment. Early intervention with parents is essential in developing effective coping skills. Parental support positively influences the child's ability to adjust to painful experiences.

### Assessment of Pain in Children

To achieve effective pain management, pain intensity and relief obtained from interventions must be assessed at regular intervals. Because children cannot, or do not, report pain consistently to health care providers, one must carry a high index of suspicion regarding the presence of pain. Pain assessment in infants and children is not necessarily straightforward. Chronologic and developmental ages influence the accurate assessment of pain. In infants, properties of their cry, facial movements, and body posturing all give clues to the presence of pain. Whereas a shrill, uncontrollable cry, contortion of facial features, restlessness, and arching of the back are indicators of pain, a silent, lethargic, submissive infant or child may also be in pain. The goal is to prevent pain from becoming severe by initiating early intervention, rather than facing the difficult task of treating established pain.

Early intervention is possible only if a thorough patient assessment is obtained. As mentioned previously, signs of pain may not be readily apparent. The nurse must use age-appropriate assessment techniques or instruments. Physiologic responses to pain are manifested by tachycardia, tachypnea, hyperventilation, hypertension, diaphoresis, and nausea and vomiting. These responses are all measurable. Self-report instruments are appropriate for use in children aged 4 years and older. These tools are in the form of graphic rating scales, visual analog scales, numeric scales, and color scales. [1,41] The use of instruments is necessary for obtaining a baseline and for periodic reassessment of pain intensity. It is important to use pain rating instruments that have been tested for reliability and validity. If the child is unable to report pain, the parent should be asked to assist in determining the presence or severity of pain by evaluating changes in behavior (e.g., eating less, playing less, crying more, or sleeping more). Once a plan of pain assessment has been established, the same methods should be used consistently. Physiologic indicators, behavioral responses, and self-report instruments should be used for reassessment at least every 2 hours after instituting interventions for pain management. [1,41]

### Procedure-Related Pain

Invasive procedures are the most painful and traumatic events experienced by children receiving treatment for cancer. [45,46,47,48,49] and [50] Aggressive treatments, such as high-dose chemotherapy, are also major sources of pain and discomfort for children. Although procedure-related pain represents an acute, brief experience, it is accompanied by fear and anxiety. Researchers have reported that bone marrow aspirations and lumbar punctures are perceived as extremely painful by children with cancer.[45,49,50] Previous studies have shown that children do not adapt to the discomfort associated with invasive procedures, but they experience greater levels of anxiety with repeated painful experiences. Children often experience symptoms such as depression, insomnia, and anorexia before the clinic or hospital visit when such procedures are scheduled.

Consensus among professionals caring for children with cancer supports a developmental approach to managing pain associated with invasive procedures. The Consensus Conference on the Management of Pain in Childhood Cancer agreed on the following principles for management of invasive procedures [51]:

1. The child and parents must be prepared, with specific methods indicated for the parent to help the child relax.
2. Treatment of pain and anxiety should be maximal for the initial procedure to reduce the development of subsequent anticipatory anxiety symptoms.
3. Staff responsible for procedures must be knowledgeable about behavioral and pharmacologic treatment of acute pain and anxiety.
4. Appropriate monitoring and resuscitative equipment must be readily available.
5. Staff must demonstrate competence in performing invasive procedures.
6. The child must be evaluated to assess effectiveness of treatment in reducing pain and anxiety.
7. As pleasant an environment as possible should be created in the treatment room.

These principles are supported in the Clinical Practice Guidelines for managing procedure-related pain published by the U.S. Agency for Health Care Policy and Research.[52]

Pharmacologic management of procedural pain should include analgesic and sedative agents. Conscious sedation is used at many institutions and is defined as a minimally depressed level of consciousness that retains the patient's ability to maintain a patent airway independently and continuously and respond appropriately to physical stimulation or verbal command.[53,54] Various pharmacologic approaches are used, most of which combine an opioid analgesic with a benzodiazepine for anxiolysis and sedation. Table 44-4 describes specific nursing responsibilities related to sedation of patients for invasive procedures.

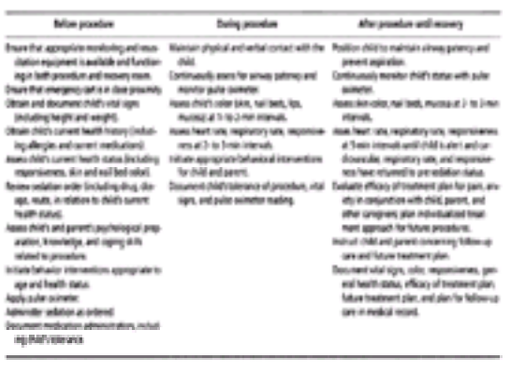

**TABLE 44-4. NURSING RESPONSIBILITIES RELATED TO SEDATION OF PEDIATRIC PATIENTS FOR INVASIVE PROCEDURES**

Topical (EMLA cream) and local anesthetics (lidocaine buffered with sodium bicarbonate) are used extensively in the management of procedure-related pain. EMLA cream, the first topical anesthetic preparation to penetrate intact skin, consists of lidocaine and prilocaine in a 1:1 ratio. The depth of anesthesia obtained from using EMLA is approximately 5 mm, and the duration of action is 4 hours. A thick layer of EMLA cream (approximately 2 mm) is significantly more effective than thinner layers. The anesthetic effects of the cream depend on thorough hydration of the skin; the skin should be covered with an occlusive dressing after application and left undisturbed for at least 1 hour.[55] A decreased effectiveness has been observed in African-American patients; this is thought to be due to the presence of a thicker stratum corneum.[55] Studies have shown that use of EMLA cream can reduce pain experienced by children undergoing venipuncture, injections through implantable catheters, and lumbar puncture.[55,56,57 and 58] For more invasive procedures such as bone marrow aspiration or lumbar puncture, the most complete anesthesia occurs 90 to 120 minutes after application of EMLA cream. Patients who are seen in the outpatient setting can have the cream applied at home to prevent delays in the clinic.

A traumatic experience during a child's first invasive procedure may affect the child's ability to cope with future procedures.[49] Parental participation is helpful to the child, particularly for toddlers and preschool children, in whom separation issues are paramount. Parents who are present during invasive procedures should receive specific information beforehand as to what will take place and suggestions as to what they can do to help their child during the procedure. Involving the parents during a procedure provides a source of support for the child. During minor procedures, such as venipuncture or intravenous access, the parent can hold and hug the child while assisting in isolating and restraining a limb or body part. During more extensive procedures (e.g., bone marrow aspiration or lumbar puncture) the parent can be positioned close to the child, within the child's view, to talk with and soothe the child. Parents who are uncomfortable in this role and who prefer not to accompany the child into the procedure room should be reassured that the child will be well treated. Another adult can assume the role of the parent in this situation.

Tactile stimulation and relaxation techniques are behavioral methods that can be used to diminish procedure-related pain as well as acute pain. An infant can be provided with a pacifier or sucrose nipple to suck during episodes of pain. Relaxation techniques reduce muscle tension, which often accompanies pain. Infants can be swaddled in a warm blanket, held securely, and rocked. Toddlers and preschool children may also benefit from being held and rocked. Older children can be instructed in relaxation techniques such as closing their eyes and deep breathing. Practicing these methods along with them reinforces these techniques.

Medical play is an innovative method used to instruct and educate the child regarding diagnostic tests and procedures. As the procedure is explained, the child has the opportunity to ask questions and to examine equipment. The child is encouraged to perform the procedure on a doll. Anxiety related to the procedure is relieved as the child gains understanding of the procedure and the sensations they will experience.

Distraction and guided imagery are two cognitive methods of nonpharmacologic pain management. Distraction involves concentrating on an event or object other than the pain. Infants and toddlers are easily distracted because of their short attention span. Older children can be distracted with activities such as video games, television, and music. Guided imagery works well with school-aged and older children who can visualize an enjoyable experience or pleasant memory. The child describes the event in detail as he or she visualizes it. The effectiveness of this method may be enhanced by the use of a coach. The coach may be a parent or other adult who discusses the event with the child and who keeps the image alive. Physical tactics may also be used as nonpharmacologic methods of pain management. Many times these are useful in older children who do not require pharmacologic management for minor procedures or discomfort. These ethods include application of heat or cold, immobilization of the affected limb or body part, or massage therapy.

### Disease- and Treatment-Related Pain

Nurses must be knowledgeable about the basic pathophysiology of cancer pain and treatment-related side effects. The World Health Organization's three-step analgesic pain ladder should be incorporated into the approach to pain management for every child with cancer.[59] Nurses must acquire extensive knowledge of common analgesics and narcotics used in pediatric pain management. Interdisciplinary pain management teams are used in numerous pediatric cancer centers. These teams serve as consultants and provide expertise in the assessment and management of pain. The nurse often serves as the coordinator of care, playing a key role in cancer pain management.

Pharmacologic management of disease-related pain involves various methods, discussed in detail in Chapter 43. More than a trial of one type of medication may be necessary to find the appropriate agent to manage a patient's pain. The route of administration must be considered as well. Providing pain relief by administering deep intramuscular injections as an alternative to the intravenous route is not appropriate therapy, because many oral preparations are now available with comparable efficacy. Nonsteroidal antiinflammatory drugs, acetaminophen with codeine, and morphine are commonly used in the management of disease-related pain.[59] All are available in the oral form, and nonsteroidal antiinflammatory drugs and morphine are available as intravenous preparations. Appropriate dosing is imperative. Doses should be titrated to increase the amount of analgesia and to minimize side effects.

### Central Line Care

Patients on prolonged or intensive treatment regimens will require a CVAD. The reasons are varied but may include administration of blood products and intravenous fluids, chemotherapy, parenteral nutrition, peripheral blood stem cell harvest and peripheral blood stem cell or bone marrow reinfusion, antibiotics, and repeated blood specimens. Several types of CVADs are available and are classified as external catheters, such as the Hickman and Broviac, or indwelling Silastic catheters, such as the Infusaport or Portacath. Chapter 12 discusses these catheters in detail. Groshong catheters are used at some institutions and do not require the instillation of heparin to maintain patency. Nurses caring for children with venous access devices must be aware of the complications related to indwelling catheters. These include infection, bleeding, thrombus formation, and catheter damage.[60] Patient and parent education regarding the care of external catheters should be based on institutional guidelines. Instruction must include a detailed discussion of sterile technique, flushing with saline and heparin when appropriate, and dressing changes. Good hand washing is imperative in preventing infection.

Standard guidelines to determine when removal of the catheter is necessary include positive blood cultures beyond 72 hours of antimicrobial therapy based on susceptibility testing or evidence of a tunnel infection,[61] catheter occlusion unresponsive to thrombolytic or chemical treatments, and a suspected or documented catheter-related infection causing septic shock.[62] When antibiotics are administered to patients with double- or triple-lumen catheters, the antibiotics must be rotated to each of the ports and lumina to avoid persistent bacterial colonization of an untreated lumen, a source of continued infection. When infections are treated and the CVAD is not removed, the length of therapy depends on the duration of bacteremia or fungemia and the immunologic status of the patient. Parenteral antibiotic therapy usually continues 7 to 10 days after the first negative blood culture is obtained in the immunocompetent patient, and 10 to 14 days in the patient who is

immunocompromised.

### Chemotherapy Administration

An understanding of the actions and side effects of specific chemotherapeutic agents is essential for nurses caring for children with cancer (see Chapter 10). Most institutions require nurses to complete a chemotherapy certification course before administering these drugs. Chemotherapy courses for nurses should include an overview of the principles of chemotherapy, classification and actions of specific agents, side effects, special considerations (e.g., interactions with other drugs), proper administration and handling, disposal of materials, and precautions to be taken with vesicants ( Table 44-5). Nurses should be observed in the administration of chemotherapy and should demonstrate competence before completion of the certification course. Specific guidelines for safe practice in the administration of chemotherapy have been established and are described in Table 44-6.[63,64]

| |
|---|
| **Before administering chemotherapy or biotherapy, the nurse should be able to do the following:** |
| Demonstrate familiarity with cancer chemotherapeutic or biotherapeutic agents (pharmacokinetics, dosage, interactions, stability, administration, side effects, toxicities, and latent effects). |
| Interpret laboratory values that determine need for delay in treatment administration or dose adjustment. |
| Plan for the management of treatment side effects. |
| Plan for potential extravasation (chemotherapy) or anaphylaxis (chemotherapy and biotherapy). |
| Initiate procedure for nursing interventions in emergency situations. |
| Verify the appropriateness of the drug dosage ordered by the physician by verifying the dosage with the protocol and confirming it with a second person. |
| Educate patients and families about the treatment. |
| **When applying knowledge in a clinical setting, the nurse should be able to do the following:** |
| Select an appropriate site for therapy; when administering vesicant chemotherapy peripherally, select an appropriate vein, perform the venipuncture, and anchor the needle safely. |
| Administer the chemotherapy and biotherapy safely according to the facility's procedure. |
| Document chemotherapy or biotherapy administration and patient's reaction to treatment. |
| Dispose of all materials and unused chemotherapeutic or biotherapeutic agents safely. |

**TABLE 44-5. OBJECTIVES FOR EDUCATIONAL PROGRAMS PREPARING NURSES TO ADMINISTER CHEMOTHERAPY OR BIOTHERAPY**

| |
|---|
| Use care and strict aseptic technique in handling chemotherapeutic agents to prevent any physical contact with the substance. |
| Prepare drugs in a properly ventilated room or biologic safety cabinet (incorporates protective front panel and vertical laminar airflow to reduce potential for inhalation during preparation). |
| Wear disposable gloves and protective clothing and discard in special container after each use. |
| Use a sterile gauze pad when priming intravenous tubing, connecting and disconnecting tubing, inserting syringes into vials, breaking glass ampules, or any other procedure in which antineoplastic drugs may be inadvertently discharged. |
| Dispose of all contaminated needles, syringes, intravenous tubing, and other contaminated equipment in a leak-proof and puncture-resistant container; do not recap or break needles. |

**TABLE 44-6. GUIDELINES FOR SAFE HANDLING OF CHEMOTHERAPEUTIC AGENTS**

Chemotherapeutic agents must be given through a free-flowing intravenous line. The infusion should be stopped immediately if any sign of infiltration occurs (i.e., pain, stinging, erythema, swelling). Agents such as vincristine, vinblastine, mitomycin-C, and doxorubicin pose significant clinical problems when they become extravasated into subcutaneous tissue.[65]

If extravasation occurs, the chemotherapy infusion should be immediately discontinued, and aspiration of any residual drug and blood should be attempted from the tubing, needle, and site. If the nurse is unable to aspirate the drug in the tubing, the needle or catheter should be removed. Direct pressure to the extravasation site should be avoided. Specific antidotes for chemotherapeutic agents are recommended and are found in Table 44-7. When an antidote is available, it should be instilled through the catheter. If the catheter has been removed, the nurse should inject the antidote into the subcutaneous tissue at the location of the extravasation using a 25-gauge needle. Warm or cold compresses discussed for use in the extravasation of specific agents ( Table 44-7) should be used for 20-minute intervals four times a day for 24 hours.[65] The affected arm should be elevated if possible for 48 hours. The site should be observed for induration, pain, erythema, swelling, blistering, and necrosis.

| Category of agent | Treatment | Antidote preparation | Local care |
|---|---|---|---|
| Alkylating agent<br>Mechlorethamine (nitrogen mustard)<br>Cisplatin | Isotonic sodium thiosulfate | Mix 4 mL of 10% sodium thiosulfate USP with 6 mL sterile water for injection; administer 2 mL into site for each milligram of drug extravasated. | Application of heat and cold not proven effective. |
| Antitumor antibiotic<br>Mitomycin C | 99% DMSO | Apply 1.5 mL topically to site q8h for 14 d; allow to air dry. | Application of heat and cold not proven effective. |
| DNA-intercalating agent<br>Doxorubicin<br>Daunomycin | 99% DMSO | Apply 1.5 mL topically to the site q8h for 14 d; allow to air dry. | Apply cold pack immediately without pressure for q8h for first 24-48 h after injury. |
| Plant alkaloids<br>Vincristine<br>Vinblastine | None available | | Elevate extremity and apply a warm (not hot) pack for 15-20 min q8h for 24-48 h after injury. |

DMSO, dimethyl sulfoxide.

**TABLE 44-7. LOCAL TREATMENT FOR CHEMOTHERAPY EXTRAVASATION**

If extravasation of chemotherapeutic agents occurs in a patient with an implanted port, a burning sensation may be experienced before swelling develops. When the patient expresses discomfort, the previously discussed guidelines should be followed. Radiologic examination to verify placement and patency of the CVAD should be performed as soon as possible.

A potentially fatal complication that can occur with certain chemotherapeutic agents is allergic reaction. Anaphylaxis can occur with L-asparaginase, bleomycin, the epipodophyllotoxins, and carboplatin. The nurse who is administering an agent that is known to cause anaphylaxis should always assess the patient for previous adverse reactions. When these agents are given, the nurse must assess for signs and symptoms of local reactions such a rash, hives, or pruritus. Systemic reactions are characterized by chest tightness, difficulty breathing, bronchospasm, wheezing, cough, chills, nausea, tachycardia, cyanosis, and anxiety. Life-threatening anaphylaxis can lead to hypotension and shock. Emergency equipment and drugs should be readily available when administering these potentially anaphylactic agents in the clinic or hospital. The following medications should be included in the emergency supplies for use in the event of an anaphylactic reaction: diphenhydramine, 1 mg per kg i.v. push (i.v.p.) (maximum, 50 mg); hydrocortisone, 2 mg per kg i.v.p. (maximum, 250 mg); and epinephrine, 1:10,000, 0.1 mg per kg i.v.p. (maximum, 1 mg per dose). The appropriate size Ambu bag and mask and supplemental oxygen should be available as well if assisted ventilation is needed.

### Planning for Care at Home

Planning for discharge to home should begin when the child is admitted to the hospital. The nurse must be familiar with resources available to assist the patient's family members in meeting their needs after hospital discharge. Coordination of care between the hospital and home is essential. Many families require home care services provided by public agencies, hospitals, or organized home care agencies. Home care nurses are generalists who provide advanced technical skills and

supportive nursing care for many different pediatric problems. Children with cancer may require hyperalimentation, i.v. antibiotic therapy, chemotherapy, central line care, pain management, and psychosocial support. Coordination of care in the home requires that the pediatric oncology team and the home care agency work together to develop and implement a plan of care.

The pediatric oncology nurse initiates the first contact with the home care nurse and provides a detailed overview of the child's diagnosis, treatment plan, and specific needs in the home setting. Answers to specific questions about the ability of the home care agency to care for children with cancer adequately should be obtained (Table 44-8). After the initial contact, a nurse from the home care agency may visit the child and family in the hospital and meet with the pediatric oncology team to determine specific interventions.

| |
|---|
| Does the agency employ nurses with pediatric experience? If so, how many nurses? |
| Do the nurses have experience with cancer patients? |
| Are the nurses certified or competent in chemotherapy administration? |
| Do the nurses have experience with long-term venous access devices (e.g., Broviac, Hickman)? |
| Can blood product transfusion be administered in the home? |
| Are the nurses familiar with side effects of cancer therapy? |
| Are appropriate precautions followed to minimize exposure to infectious organisms? |
| What kind of coverage is provided (i.e., 24 h?) |

**TABLE 44-8. ASSESSMENT OF HOME CARE SERVICES FOR A CHILD WITH CANCER**

### Providing Information on the Disease and Treatment

The pediatric oncology nurse works with other members of the interdisciplinary team to provide education for the family and patient regarding the diagnosis, treatment, and psychosocial issues of childhood cancer. At the initial diagnostic talk, the nurse should be present. During subsequent discussions, the nurse reinforces and reviews information and answers the questions and concerns of the patients and family. Most pediatric cancer centers have structured education programs for families that include written materials, handbooks for parents, formal teaching sessions, videotapes, tours of the hospital and clinic, and structured play activities for the child to learn more about the illness and treatment. Parent and patient education is ongoing and continues throughout treatment and after completion of therapy.

Educational programs for families and patients should include discussion of the pathophysiology of the different types of cancer, various treatment options, general side effects of treatment, growth and development concerns, and family issues. Parents must become familiar with the special needs of their child. Psychomotor skills, such as central line care and subcutaneous injections, may need to be acquired by the parents and child and are taught by the nurse before hospital discharge. [1] Assessment and evaluation of specific teaching given to families and patients should be documented in the patient's record. The nurse should be aware of the numerous educational resources that exist for children and families with cancer. Organizations that provide these materials are found in Chapter 57.

Nurses caring for children with cancer must understand that patient education must relate to the child's developmental stage. Knowledge of growth and development helps the nurse to communicate with the child before painful procedures or unfamiliar tests and to understand the child's uncertainty about hospitalization. For example, when dealing with a preschool child, it may be more appropriate to use medical play to demonstrate how a bone marrow aspiration is performed. In comparison, the adolescent needs a detailed discussion of the procedure, a description of the experience, and instructions about what to do. Chapter 57 describes specific considerations for discussing the disease and treatment with children at various ages.

### Facilitating Psychosocial Support

Nurses work with the interdisciplinary care team to provide psychosocial support for the patient and family. The nurse must understand family systems and the role each member plays in the family. The expert nurse considers cultural influences on the child and family and conducts a detailed assessment of the family's support system, including their strengths and weaknesses. Evaluation of community resources is an integral part of the family assessment.

Diagnosis, treatment, discontinuation of therapy, relapse, and terminal illness are crisis points for families who have a child with cancer. [66] At the time of diagnosis, anxiety, fear, anger, and depression are commonly felt by family members. Coping with the crisis of childhood cancer requires that the family develop new attitudes, behaviors, and coping techniques. Nurses provide support by helping family members work together. During the treatment period, nurses are resources for the family, teaching them about the disease and treatment and providing support during stressful situations. Nurses play a major role in preventing parental overprotection and isolation of the child. Families must be reassured that maintaining discipline during treatment is in the best interest of the child. Many children who are diagnosed with cancer are successfully treated and go on to lead normal adult lives. Maintaining discipline during childhood enables them to become well-adjusted adults. Essential coping tasks for the family during the diagnostic period and initiation of treatment are described in Table 44-9.

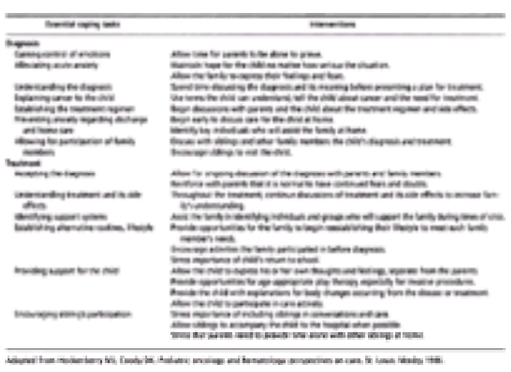

**TABLE 44-9. FAMILY COPING TASKS AND INTERVENTIONS DURING DIAGNOSIS AND TREATMENT**

Over time, the family ideally learns to adjust to the changes brought on by treatment of childhood cancer. Cessation of therapy can bring with it new fears for the child and family. Preparation for discontinuation of therapy is as essential as the education provided at the time of initial diagnosis. Nurses must encourage the parents to verbalize their fears. Table 44-10 identifies specific interventions for families at the time of discontinuation of therapy.

| | |
|---|---|
| Adapting to discontinuing treatment | Begin discussions several months before therapy is discontinued.<br>Allow time for parents and child to ask questions and verbalize concerns. |
| Recognizing fear of relapse | Discuss openly the possibility of relapse.<br>Review concerns for parents and child regarding what to expect if relapse should occur. |
| Realizing impact of parent's attitude | Discuss importance of parents' attitude and how it affects the child.<br>Stress importance of verbalizing fears while recognizing the positive situation of discontinuing therapy. |
| Supporting child's needs and preventing fears | Reassure the child that therapy would not be discontinued unless he or she was doing well.<br>Allow the child to express fears separately from parents (provide support and stress need for courage and trust). |

Adapted from Hockenberry MJ, Coody DK. Pediatric oncology and hematology: perspectives on care. St. Louis: Mosby, 1986.

**TABLE 44-10. FAMILY COPING TASKS AND INTERVENTIONS AT DISCONTINUATION OF THERAPY**

Recurrent disease brings with it a crisis for the entire family. Adequate time must be spent in counseling and providing support during the period when families must face the failure of treatment. Parents frequently feel guilty, and they may be angry. Parents may question why the disease has recurred in spite of all they have done. Nurses caring for children and families experiencing a relapse must be excellent listeners and must create a caring atmosphere amid the turmoil. Table 44-11 reviews specific nursing interventions for families during this time.

| Essential coping tasks | Interventions |
|---|---|
| Alleviating initial shock of relapse | Allow parents to express shock and disbelief.<br>Provide time for grieving before initiating discussion of treatment plan. |
| Understanding the impact of relapse | Discuss the seriousness of relapse, yet provide hope in the situation.<br>Offer facts regarding possible outcome of the disease. |
| Discussing relapse with the child | Express importance of being truthful with the child.<br>Discuss the relapse with the child and the need to begin therapy again (realize the child will perceive the seriousness of the situation by observing parents and staff). |
| Expressing appropriate feelings of grief | Encourage expression of feelings and need for family to maintain a realistic outlook toward the situation.<br>Identify key support individuals to maintain close follow-up with all family members. |

Adapted from Hockenberry MJ, Coody DK. Pediatric oncology and hematology: perspectives on care. St. Louis: Mosby, 1986.

**TABLE 44-11. FAMILY COPING TASKS AND INTERVENTIONS WITH RELAPSE**

At the time of the initial cancer diagnosis, families with a child who has cancer are confronted with the possibility of death. Return of the disease brings with it the realization that the child may not survive. Parents facing the loss of a child to cancer have been through numerous crises since diagnosis but none so difficult as the awareness that their child may die. Nurses can assist the family during this crisis by helping to identify strengths that will support them throughout this difficult time. Nursing interventions that promote effective coping tasks include helping the family accept the terminal status of their child's disease, allowing the family to participate in the child's care as much as possible, including hospice support (see Chapter 51), encouraging expression of emotions and guilt feelings, and planning for the future (Table 44-12).

| Essential coping tasks | Interventions |
|---|---|
| Accepting the child's impending death | Allow the parents to verbalize their fears of the child's death.<br>Discuss any questions they may have to decrease their worries and concerns.<br>When possible, listen to the parent's wishes and demands. |
| Participating in the child's care | Encourage the family to remain involved with the child's care. (This involvement allows them to comfort the child while giving the parents a sense of belonging and need; it will also assist in preparing them for the inevitable loss.) |
| Expressing appropriate emotions | Stress the importance of expressing grief.<br>Identify key individuals who will provide comfort and reassurance.<br>Encourage relatives and significant others to be involved to give parents an opportunity to rest and maintain physical strength. |
| Resolving guilt feelings and sense of helplessness | Reassure parents that they could have done nothing to prevent the child's death.<br>Assure them that they are doing everything possible by providing comfort and support.<br>Stress that the most important role is their presence with the child. |
| Planning for the future | Stress the need for the family to look toward the future. |

Adapted from Hockenberry MJ, Coody DK. Pediatric oncology and hematology: perspectives on care. St. Louis: Mosby, 1986.

**TABLE 44-12. FAMILY COPING TASKS AND INTERVENTIONS DURING END-STAGE DISEASE**

### Promoting Normal Growth and Development

Cancer therapy has the potential to cause significant developmental and growth delays. [67] The nurse must continually assess the child's growth and development during treatment and after cessation of therapy. Evaluation of the child's weight and height should be documented on standardized growth charts at regular intervals. Children younger than 3 years should have head circumferences documented. Changes in weight or lack of expected growth in height or weight should be followed closely. Any percentile change on the growth chart or weight loss of 5% or more should be evaluated. [68,69] Specific nutritional interventions described in Chapter 42 should be started immediately once changes in weight occur.

Nurses can facilitate normal development by ensuring that the child is treated at an age-appropriate level when visiting the clinic or hospital. The importance of meeting basic needs and supporting developmental tasks should be emphasized at each visit. Family support should be given to encourage normal childhood development at home. Table 44-13 describes specific nursing interventions designed to promote developmental tasks for children with cancer. Accurate assessment of any disruption in growth or development allows for early intervention.

**TABLE 44-13. MEETING CHILDREN'S BASIC NEEDS AND SUPPORTING DEVELOPMENTAL ISSUES**

Returning to school is an important milestone for children with cancer. Every attempt must be made to ensure that the child has the opportunity to return to the classroom despite the disease and treatment. Chapter 50 discusses the importance of school reentry programs for children with cancer. Nurses are instrumental in assisting with the child's return to school.[70] Visits to the school by the nurse to meet with teachers and to talk to the child's peers are commonly offered by most comprehensive childhood cancer centers. At times, a child with cancer must refrain from returning to school, often because of the intensity of the treatment program. When a child must have homebound instruction before returning to the classroom, ongoing communication between the child and classmates should be encouraged. Successful school reentry is a goal for all children and must be perceived as such by all members of the patient's care team. The nurse must be vigilant in attempting to help the child return to school when possible.

### Following Up Long-Term Survivors

Nurses who provide care for survivors of childhood cancer must understand the late effects of therapy and should have an extensive knowledge of normal growth and development. Current collaborative practice models designed to follow these patients include nurses as direct care providers. These models provide a more comprehensive approach to meeting the complex needs of children and adolescents who have survived cancer. Nurses, usually in advanced practice roles, are able to perform physical assessment, to provide growth and development evaluation, and to conduct screening tests for specific late effects related to the type of cancer or its treatment.[71] A major aspect of the nursing role is patient and family education regarding possible late psychological or physical consequences of childhood cancer. Chapter 49 discusses specific late effects in detail.

Nurses must be knowledgeable regarding the long-term complications of specific chemotherapeutic agents. Toxicity related to therapy can result in long-term disability. Anthracyclines, which include doxorubicin and daunorubicin, can produce irreversible cardiac damage.[72,73] Cumulativedoses of anthracyclines must be closely monitored by the nurse, and examination of left ventricular function should be ordered periodically as part of the overall medical management plan (see Chapter 10). Agents known to cause renal or bladder complications include cisplatin, ifosfamide, and cyclophosphamide.[74] Long-term kidney damage can occur with other agents as well. Nurses must be aware of these complications and should monitor the patient for signs of bladder toxicity such as hemorrhagic cystitis after cyclophosphamide therapy. Renal function should be evaluated by obtaining serum chemistry determinations on each return visit. Children who have had bone marrow transplantation have the potential for developing long-term pulmonary toxicities and must be evaluated periodically for signs and symptoms or respiratory compromise.[75] These individuals should remain nonsmokers after transplantation and be treated aggressively for respiratory illnesses. The nurse should assess the patient for symptoms such as dyspnea, shortness of breath, cough, or fever. Chest radiographs and pulmonary function tests should be performed routinely in these individuals. Several agents, such as methotrexate, chlorambucil, 6-mercaptopurine, daunorubicin, and doxorubicin, are associated with long-term liver toxicity.[76] Nurses must be aware of the potential for development of hepatitis, hepatic fibrosis, and cirrhosis in these patients. Gastrointestinal toxicities are most frequently caused by combined chemotherapy and radiation therapy. Signs and symptoms include abdominal pain, nausea, vomiting, diarrhea, constipation, and gastrointestinal bleeding. Bone growth is usually not affected by chemotherapy alone; however, prolonged use of methotrexate and corticosteroids may cause osteoporosis, bone pain, and increased susceptibility to fractures.[77] Nurses must be comprehensive in their assessment for musculoskeletal complications in children after treatment of cancer.

Nurses caring for survivors of cancer who received cranial irradiation and intrathecal methotrexate at a young age must be aware of the possible late effects of this treatment. Intellectual and motor function may be impaired because of interference with neural development before maturation of the brain is complete.[78,79] Memory loss may occur in children receiving high doses of irradiation. Children younger than 3 years are at the highest risk of this complication.[80] Assessment of these children must include an extensive neurologic evaluation that includes cognitive function. Nurses should assess school attendance and performance because problems with mathematics and reading may occur.

Radiation therapy can cause bone growth to cease and can decrease the function of reproductive glands responsible for manufacturing growth hormones.[81,82] Nurses must document growth by assessing height and weight at each visit. Changes in growth velocity should be referred for further evaluation. Further assessment must include measuring parental heights, obtaining a radiograph of the patient's left wrist to predict further growth potential, and assessing gonadal development and pituitary function. An endocrinologist should be consulted when abnormalities are found or are suspected.

Knowledge of the effects of radiation therapy and alkylating agents on hormonal function, fertility, and sterility is important for the nurse caring for the cancer survivor. The potential for gonadal dysfunction depends on the child's age at the time of diagnosis, the child's sex, the type of treatment, and the duration and total dose of treatment.[83] Nursing assessment must include careful documentation of sexual development using the Tanner staging scale and a detailed history.

Survivors who have undergone radiation therapy to developing bone or cartilage need close observation of the irradiated bone to detect abnormalities such as spinal kyphosis or scoliosis, leg-length discrepancy, and skull or facial disfigurement. Because irradiated bones are more fragile, the survivor is at risk for bone fractures, often has functional limitations, and heals more slowly in the presence of infection. Osteoporosis may develop.[77] Children who have received irradiation to the mandibular area are at risk for dental caries, arrested tooth development, and incomplete dental calcification. A complete assessment of the oral cavity at every clinic visit is essential in children who have received irradiation to the mandible.

## PROFESSIONAL DEVELOPMENT

Pediatric oncology nurses must continue to pursue ways to maintain their professional competence. Participation in professional organizations, such as the Association of Pediatric Oncology Nurses, ensures ongoing involvement in continuing education, professional development, and research. Pediatric oncology nursing certification is available through Oncology Nursing Certification Corporation. Nurses who successfully pursue certification in pediatric oncology demonstrate a commitment to the specialty and obtain the credentials associated with specialization. Pediatric oncology nurses must continue to be committed to their colleagues as well as to the ongoing development of collaborative relationships. Through these relationships, the role of the nurse in pediatric oncology can be realized.

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# REHABILITATION OF THE CHILD WITH CANCER

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## INTRODUCTION

The comprehensive rehabilitation of children with cancer requires an interdisciplinary team approach across the continuum of care. Extraordinary improvement in survival of children with a wide variety of cancer types is the result of advances in treatment discussed previously in this text. Accompanying this success are new challenges resulting from morbidity in the survivors, due to the cancer itself or to its interventions (see Chapter 49). Physical, psychological, and social function may be affected. Minimizing the consequences of these sequelae on future quality of life is the overall goal of rehabilitation.

The focus in rehabilitation is typically on disability management. Dimensions of disablement and functioning include *impairment*, *activity*, and *participation*.[1] *Impairment* refers to the loss or abnormality of psychological, physical, or anatomic structure or function and applies to the organ system level of function. Specific impairments, in this case related to different types of cancer, may have an impact on the child's age-appropriate activity, affecting mobility, self-care, communication, cognition, or psychological and social function. *Disability* is limitation in activity, in the manner or within the range considered normal, due to impairment. The same impairment may or may not result in activity limitations in different children. *Handicap* exists when an impairment or disability restricts participation in a role that is normal for age and gender within the social and cultural milieu. Participation restrictions are external to the individual, such as those imposed by architectural or attitudinal barriers.

Goals of pediatric disability management include minimizing the impairment and maximizing activity and participation in age-appropriate life roles: school, play and recreation, and work. Major objectives include facilitating independent child function in each domain that is affected and minimizing the burden of disability for the parents and caregivers. Function is promoted in mobility, self-care, communication, cognition, or psychosocial domains. Efforts are directed toward achieving maximum independence despite the disorder, primarily through six categories of intervention strategies to help mitigate disability. [2] These include (a) preventing or correcting additional secondary disability; (b) enhancing function in the affected system; (c) enhancing function in unaffected systems; (d) using adaptive equipment to promote function; (e) modifying the social and vocational environment; and (f) using psychological techniques to enhance patient performance and patient and family education. In pediatric rehabilitation, prescriptions for therapy programs, adaptive equipment, orthoses, and prostheses must be appropriate to the age and developmental level of the child and include considerations related to ongoing growth and development. [3,4]

The interdisciplinary pediatric rehabilitation team evaluating and addressing individualized goals for the child with a cancer-related disability might include one or more of the following specialists: pediatric physiatrist (specialist in physical medicine and rehabilitation), rehabilitation nurse, physical therapist, occupational therapist, speech-language pathologist, psychologist, social worker, therapeutic recreation specialist, prosthetist-orthotist, special educator, and vocational counselor. Care should be coordinated, comprehensive, and family-centered. The pediatric rehabilitation team must work in close collaboration with the pediatric oncology care team.

Significant functional gains follow rehabilitation of adult patients with cancer. [5] Although evidence for similar gains after rehabilitation of the pediatric cancer population has not been directly evaluated, extrapolation from adults with cancer and from children with other disabling conditions supports the provision of pediatric rehabilitative services to children with cancer.

The first section of this chapter focuses on the common issues of limitations in activity and restrictions in participation that cross malignancies involving different organ systems. The second section focuses on specific functional limitations associated with the major types of pediatric cancer that result in a significant incidence of disability. Finally, critical-illness polyneuropathy and myopathy, although not unique to children with cancer, are seen with sufficient frequency in this population and with such significant impact on function as to warrant inclusion.

## REHABILITATION PROBLEMS

### Mobility

Deficits in functional mobility may occur with the generalized deconditioning associated with prolonged or chronic illness and immobility. Immobility is discussed in further detail in the next part of this section. As it is a highly significant issue in the pediatric cancer population. Due either to the tumor or its treatments, central neurologic involvement of the motor strip, long tracts, basal ganglia, cerebellum, or spinal cord, or peripheral neurologic involvement can result in problems in motor function due to weakness or paralysis, spasticity, or deficits in balance and coordination, often associated with limited endurance. Physical and occupational therapists work with the child and family to ameliorate specific motor deficits, prevent secondary complications such as contractures, provide training in compensatory strategies, limit use of abnormal movement patterns, and use orthotic and assistive devices as appropriate.

Progression of ambulation retraining may involve use of gait aids, often beginning with those providing more support, such as a walker, and weaning to crutches and possibly to canes before independence is reestablished. Bracing, most typically with ankle-foot orthoses, may be indicated. Design of the ankle-foot orthosis is determined by multiple factors, including ankle strength, range of motion, presence of spasticity, and mediolateral ankle stability. Examples of commonly used designs appropriate for children with cancer are illustrated in Figure 45-1. Alternative means of mobility to ambulation may be indicated, temporarily or permanently, for some children with various types of cancer. Most children who are not ambulatory require a manual wheelchair, although some benefit from use of a power wheelchair for independence in mobility (Fig. 45-2).

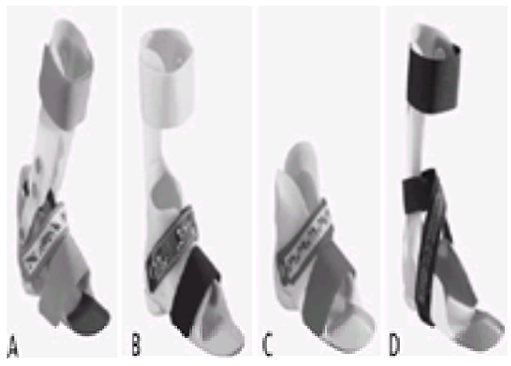


**FIGURE 45-1.** Common ankle-foot orthosis (AFO) designs appropriate for children with cancer. **A:** A hinged AFO allows dorsiflexion of the ankle and blocks plantar flexion and may be appropriate for the child with active ankle dorsiflexors and plantar flexor spasticity. **B:** Extending the trim lines anteriorly provides more mediolateral stability for a child who has excessive pronation or supination and limited control of ankle dorsiflexion and plantar flexion. **C:** A supramalleolar orthosis provides some mediolateral support for a child who excessively pronates or supinates but has adequate control of ankle dorsiflexion and plantar flexion. **D:** Lightweight dorsiflexion assistance is especially appropriate for the child with distal weakness and low tone resulting in footdrop, such as in peripheral neuropathy. This design also is known as a *posterior leaf spring*. (Photos courtesy of Cascade DAFO, Inc.)

**FIGURE 45-2.** Powered mobility adapted with a swing-away joystick and mechanism for height adjustability facilitates a child's function in a variety of natural environments. A child can rise to table level to eat with adults or lower to eye level of other children at child-sized furniture, such as preschool tables, or to the floor. Alternative keyboards, such as expanded membrane keyboards, facilitate developmentally appropriate computer activities. The equipment shown here may be appropriate for a child with a cervical spinal cord tumor, brain tumor, or critical-illness polyneuropathy. (Courtesy of Claire Morress, OTR/L, Assistive Technology Services, United Cerebral Palsy Aaron W. Perlman Center, Affiliate of Children's Hospital Medical Center, Cincinnati, Ohio.)

As endurance improves, intensity of exercise regimens can be increased. Exercise programs for children in the acute phase of their management may focus initially on passive range of motion to maintain joint flexibility. As the child is able, active participation is progressively increased, with particular activities selected to focus on the child's specific individualized therapy goals, which are advanced as possible. Participation in sports and recreational activities should be encouraged whenever possible. Owing to buoyancy and elimination of gravity, aquatic therapy often allows movement that is not possible out of the water. Again, progression can occur as the child is able, often to the level of competitive sports participation in the long term. Anthracycline, which can have lifelong effects on cardiac function, is used in more than 50% of children with cancer. Therefore, children who have received this chemotherapeutic agent should be thoroughly evaluated by a cardiologist before they engage in competitive sports.[6]

**Immobility**

Prolonged bed rest and immobility affect almost every organ system and can negatively affect a child's functional capacity. Muscle strength and endurance decrease owing to the inactivity and reduced force of gravity associated with bed rest. With complete bed rest, a muscle loses 1.0% to 1.5% of its strength per day or 10% to 15% per week.[7] Immobilized muscles have also been shown to experience a more rapid depletion of glycogen and an increased production of lactic acid during work. [8] Muscles immobilizedin a shortened position are also at risk for contracture, associated with segmental necrosis, disorganization of myofibrils, and a reduction in the number of sarcomeres.[9] Connective tissue contracts and reorganizes within 1 week of a joint becoming immobile, further increasing the risk for contracture development. Inactivity also results in increased bone resorption that may result in osteoporosis and risk for pathologic fractures. [10]

Immobilization also has significant effects on the cardiovascular system. Increased sympathetic activity leads to an increased heart rate. [11] Cardiac output, stroke volume, and left ventricular function decline, and orthostatic hypotension increases. [12] A decrease in cardiac output combined with a peripheral oxygen use deficiency causes a decline in maximal oxygen consumption.[13] Blood volume decreases with prolonged bed rest. Plasma volume decreases more than does red blood cell mass, resulting in increased blood viscosity. [13] Increased blood viscosity combined with immobility places the patient at increased risk for deep venous thrombosis.

Respiratory complications of immobility can be life threatening. Potential changes include diminished diaphragmatic movement in the supine position, decreased chest excursion, and decreased range of motion of the costovertebral and costochondral joints. These changes can result in a decrease in the vital capacity and functional reserve capacity of 25% to 50%.[13] The ventilation-to-perfusion ratio may be altered in dependent areas of the lung, resulting in arteriovenous shunting and reduced oxygenation.[14] Impaired ability to clear secretions can lead to atelectasis and an increased risk of pneumonia.

Immobility can also negatively affect metabolism and the endocrine system. A decrease in total body sodium is associated with diuresis during early bed rest. Potassium levels also decrease during the early stages of immobility. [13] Serious electrolyte abnormalities, however, rarely occur with the exception of hypercalcemia, most commonly seen in patients with high bone turnover, such as children and adolescents.[14] Glucose intolerance and nitrogen loss due to an increase in protein catabolism also are not uncommon in immobilized patients.[14]

Pressure ulcers are another complication of immobility that can occur when external pressure exceeds capillary pressure for prolonged periods. Poor nutrition, moisture, insensate skin, and shear forces are additional risk factors for skin breakdown. Supine patients are at risk for pressure ulcers over the occiput, sacrum, and heels; patients lying on their sides are at risk for breakdown over the greater trochanters; and patients who sit for prolonged periods are at risk for ulcers over the ischial tuberosities.

**Activities of Daily Living**

All members of the rehabilitation team, but specifically the occupational therapist, work with the child to increase independence in age-appropriate daily care activities such as eating, grooming, bathing, toileting, and play, with the use of adaptive equipment as needed. Bladder and bowel dysfunction, in particular, pose common special management challenges and so are discussed in detail. Family education is provided, and family members are encouraged to allow the child to function at the highest level of independence at which he or she is capable.

***Bladder Dysfunction***

Cyclophosphamide and ifosfamide, both used widely in the treatment of solid tumors, are metabolized to a urotoxic compound, acrolein, which can accumulate in the urine to levels sufficient to injure the bladder epithelium. This results in a hemorrhagic cystitis with hematuria and irritative voiding symptoms. Potential sequelae include bladder fibrosis, reflux hydronephrosis, and loss of renal function. Recently, the concomitant administration of the uroprotectant 2-mercaptoethane sulfonate

sodium (mesna) has dramatically decreased the incidence of hemorrhagic cystitis and has prevented recurrence or exacerbations of cystitis in children for whom continued administration of these agents is indicated. Mesna is excreted primarily by the kidneys, where it binds to the acrolein and renders it inert. Other prophylactic measures include hydration before, during, and after chemotherapy and urine acidification. [15]

Various combinations of urinary storage and voiding impairment may occur with tumors along the neural axis from the pons to the cauda equina, owing to lower urinary tract dysfunction. Incontinence with lesions above the level of the pons usually is due to disinhibition. Neural pathways that modulate bladder function traverse the length of the spinal cord between the pons and the sacral spinal cord, with events coordinated in the pontine micturition center. Interruption of these pathways results in storage or voiding dysfunction or detrusor-sphincter dyssynergia when there is loss of the coordinated function of the detrusor and the external striated urethral sphincter. Lesions at the level of the pons may also impair the coordinated functioning of the lower urinary tract. As seen in spinal cord lesions, manifestations usually relate to the upper motor neuron, with involuntary detrusor contractions, nonrelaxation of the external sphincter during detrusor contractions, and subsequent development of bladder wall thickening, trabeculations, and decreased compliance and storage capacity owing to detrusor hyperactivity.

Videofluoroscopy is the most informative study by which to evaluate children with neuropathic vesicoureteral dysfunction. This study superimposes pressure measurements on the simultaneous fluoroscopic appearance of the bladder and urethra and provides valuable information on the function of the bladder and urethra during filling and voiding. Clean intermittent catheterization is an effective method of bladder emptying and may be used alone or in combination with anticholinergic or a-adrenergic medications to protect the upper tracts while achieving satisfactory continence.

#### *Bowel Dysfunction*

Constipation, defined as infrequent, excessively hard and dry bowel movements, is a common problem for children with cancer. Decreased rectal filling or emptying may be due to poor intake, dehydration, decreased activity, narcotic analgesics, tumor-related neurologic injury, or neurotoxic chemotherapeutic agents. Vincristine and vinblastine are neurotoxic alkaloids, which commonly disrupt bowel function via their neuropathic effects, including peripheral neuropathy. Nonfunctional afferent and efferent pathways from the sacral cord result in impaired rectal emptying similar to that seen in neurogenic bowel due to spinal cord injury.

Management includes promoting mobility, providing appropriate positioning for the nonambulatory child, increasing dietary fiber and fluid intake, and minimizing use of medications that decrease gastrointestinal motility, if possible. Fluid intake is critical, particularly when strategies to address the constipation include the addition of bulk to the diet. Bulk without adequate hydration increases the risk of impaction. If activity is limited or if ongoing administration of narcotic or neurotoxic agents is required, a stool softener or mild laxative may be given, with adjustment of the dose and frequency to ensure good bowel evacuation at least every other day. The goal of a bowel program is to have the bowel empty regularly and adequately.

The child with a neurogenic bowel may require a formal bowel program with digital stimulation or a suppository to encourage evacuation. The presence of an anal wink indicates an upper motor neuron lesion and a better prognosis for continence, as sphincter tone usually is adequate to retain the stool. When this reflex is absent, as in lower motor neuron lesions, the sphincter may be flaccid and the patient unable either to expel or to retain feces. Constant stool leakage can occur. Because routine and consistency are critical to a successful bowel program, a convenient, relaxed time should be selected for performing the bowel program daily and should vary as little as possible from day to day. Trying to evacuate the bowels 30 minutes after a meal will take advantage of the gastrocolic reflex.

Problems with bowel management may represent a significant source of emotional turmoil for both the child and his or her family, particularly in the older, previously continent child. Successful bowel management enhances the potential of the child to achieve satisfactory independence and social acceptability.

### Communication

Children with cancer may experience communication disorders as a consequence of their primary disease, particularly with primary brain tumors, central nervous system (CNS) metastasis, or as a late effect of cranial irradiation. Depending on the area of the brain affected and the age of the child, communication may be impaired owing to deficits in speech, language, cognition, memory, or personality. Whereas speech and voice problems are related to motor dysfunction, either due to weakness of the involved structures or incoordination, language skills are more reflective of cognitive functioning. For this reason, language processing may be further compromised by concomitant impairments in critical cognitive or information-processing skills, such as memory, perception, attention, or organization, as well as behavioral impairments such as disinhibition, poor self-monitoring, limited frustration tolerance, or poor judgment. [16] Expressive or receptive language may be involved, affecting spoken or written skills (or both).

In older children with established language skills, the processing and use of language usually is abruptly disrupted. The pattern of speech and language deficits depends on the area of the brain injury. These may be related to receptive or expressive language problems or motor dysfunction, including dysarthria with weakness of the oral musculature, apraxia due to motor incoordination, or phonation deficits due to velopharyngeal insufficiency or vocal cord paralysis. The child with severe expressive language deficits and good comprehension may benefit from an augmentative or alternative communication system.

Pragmatics may be a problem when there is frontal or right hemisphere involvement. In the very young child who has not yet fully developed language skills, the pattern of language dysfunction is less predictable than that seen in the older child with a similar lesion. The very young child may present with a developmental language disorder, either secondary to specific neurologic involvement or as a part of the global developmental delay often seen in children with serious and chronic illness early in life. Little in the literature addresses the treatment-related effects on neuroplasticity in this setting and, unfortunately, owing to their limited former skill acquisition, these children have few compensatory strategies available to them. As language development generally parallels cognition, factors that affect cognition will have a similar impact on language skills. After evaluation, communication deficits may be addressed in individual or group therapy, with functional communication goals addressed within a developmental format.

Thorough speech and language assessment should be completed by the speech pathologist, and a therapeutic program should be planned that addresses communication deficits in a manner appropriate to the child's age and medical condition. For the child who has lost the capacity for verbal communication, some form of functional communication should be introduced as a means of self-expression and to indicate needs. Simple communication boards, electronic augmentative communication devices such as speech synthesizers, writing, keyboards, and sign language are among the available options.

### Cognition

Cranial irradiation, as well as intrathecal and high-dose intravenous methotrexate, has long been associated with leukoencephalopathy and learning disability. [17] Also, studies have suggested that the administration of high-dose methotrexate potentiates the deleterious effects of cranial irradiation on cognition. [18] The impact on cognitive function is an often devastating late effect of cancer therapy that can significantly impair quality of life, particularly in very young children. A patient's age at the time of treatment is a major factor in the development of cognitive decline after cranial irradiation. Children with leukemia, lymphoma, or brain tumors treated before 4 or 5 years of age are at higher risk for cognitive impairment as compared to older children. [17] The deleterious effects can have a major influence on intellectual and academic performance, social competence, behavior, and vocational potential. Cognitive dysfunction in children who survive brain tumors is covered further later in this chapter.

### Psychosocial Aspects

Issues related to psychological adjustment to chronic illness and disability, family adaptation, and sibling adjustment are critical to the long-term outcomes of children with cancer. A crucial component of a comprehensive rehabilitative program for the child with cancer, in addition to efforts directed to maximizing independent function in the domains just discussed, is consideration of school reentry and eventual work entry, as well as inclusion with the family and peers in social and recreational activities. These issues are covered extensively in Chapter 50 and Chapter 53.

## REHABILITATION ISSUES IN SPECIFIC CHILDHOOD CANCERS

### Brain Tumors

Intracranial tumors represent the second most common type of childhood cancer, with peak incidence in early childhood. [19] Children with brain tumors experience significant functional deficits related to the primary disease process and as a consequence of its treatment. As in adults, childhood brain tumors represent a

heterogeneous group of lesions that vary in pathologic characteristics, tumor biology, response to therapy, anatomic location, and age at diagnosis. With the advances in diagnostic strategies, neurosurgical techniques, and cooperative therapeutic trials over the last 30 years, more than 50% of children with brain neoplasms now are surviving. With this improved survival has come increased recognition of the significance of the long-term sequelae of the tumor, its treatment, and the impact of consequent functional deficits on quality of life.

For most brain tumors of childhood, the cornerstone of management involves surgical debulking, which then is followed by irradiation or chemotherapy (or both). The most favorable outcomes for children with neoplasms of the CNS are for those with cerebellar astrocytomas. The worst outcomes are among those children with brainstem gliomas, ependymomas, and supratentorial primitive neuroectodermal tumors. It is noteworthy that the 5-year survival for children with medulloblastoma has improved dramatically with routine use of craniospinal irradiation. In general, children younger than 2 years have a worse survival rate and significantly increased morbidity in comparison to older children. A more comprehensive discussion of specific tumor types, epidemiology, treatments, and outcomes is included in Chapter 27.

Each treatment modality may be associated with both transient and long-term effects, which may have an impact on duration of survival, functional outcome, and quality of life.[20] Despite significantly decreased surgical morbidity due to improvements in surgery, anesthesia, and postoperative care, surgical resections may be associated with significant neurologic morbidity related to the age and preoperative clinical status of the child, type of tumor, location, and extent of resection. [21]

Although the introduction of radiotherapy has significantly improved duration of survival in a number of people with CNS tumors, it is well recognized that cranial irradiation has long-term effects that are progressive and potentially devastating. Radiation myelitis with spastic paraplegia or quadriplegia may result from spinal cord irradiation. Additionally, radiation to the vertebrae in the young child increases the risk of scoliosis and kyphosis. The extent of the radiation-induced injury is directly related to radiation dose, volume of CNS rradiated, and age of the child. Histologic changes in the brain after cranial irradiation may include neuronal dropout, gliosis, and proliferative and sclerosing angiopathy.[22] Long-term complications associated with these changes include cognitive deficits, endocrinopathies, vasculopathies, hearing loss, radiation necrosis, and second primary neoplasms.[23,24]

Effects of chemotherapy on the developing nervous system are less well understood. Leukoencephalopathy is a late complication associated with both radiotherapy and chemotherapy, particularly methotrexate given intrathecally or in high doses intravenously. This condition is characterized clinically by dementia, ataxia, and focal motor deficits and can progress to coma and death. Other delayed toxic effects of chemotherapy that significantly affect function include peripheral neuropathies, myopathies, and hearing loss.

Children with brain tumors may experience a wide range of functional deficits related to the effects of the primary lesion or treatment complications. These include motor, sensory, and speech and language dysfunction, cognitive impairment, and psychoemotional disorders. The nature and extent of impairment depend on the age and developmental level of the child at time of diagnosis, the location of the lesion, and the degree of neurologic compromise. With the exception of cognition, information related to rehabilitation issues is extremely scarce in the literature.

The team approach, as outlined at the beginning of this chapter, applies to rehabilitation of children with brain tumors as well. It is important for the rehabilitation team to work in close coordination with the neuro-oncology team. In the initial period after a diagnosis of CNS tumor in a child has been made, the role of rehabilitation depends on the age of the child, severity of illness, degree of functional impairment present, and management planned. Particularly if surgery is delayed or not recommended and initial management will include observation or is limited to chemotherapy, radiotherapy, or both, functional deficits should be addressed immediately. Depending on the child's clinical status, appropriate family education, support services and therapy, and adaptive equipment should be provided, with goals of decreasing the burden of care, limiting secondary complications, and optimizing age-appropriate function.

For the child who is receiving rehabilitation services while undergoing radiotherapy or chemotherapy, good communication and cooperation between the rehabilitation and neuro-oncology teams are critical. The rehabilitation team must be aware of the treatment planned and any potential complications. They must be sensitive to problems of pain, nausea, anorexia, constipation, and poor endurance, which may limit the child's ability to participate fully in the rehabilitation process. When pain is an issue, adequate pain management must be provided, including medication and adjunct strategies and services such as counseling, relaxation techniques, therapeutic modalities, and biofeedback when appropriate. Rehabilitation schedules should be modified appropriately to accommodate the antitumor treatments and allow rest periods. The occupational therapist can address energy conservation strategies and pacing with the family and child. Additionally, the team must be vigilant for subtle signs and symptoms of treatment complications or disease progression and address these with the neuro-oncologist.

Aside from the disease process itself, both radiotherapy and chemotherapy can have a deleterious effect on appetite, with dire nutritional consequences. Poor nutrition can impair wound healing and growth, diminish overall well-being, and reduce the ability to participate in age-appropriate activities and in the overall rehabilitation process. Nutritional status should be closely monitored and deficits aggressively addressed. In some cases, hyperphagia may occur, with the potential for rapid weight gain. Nutrition issues are addressed in more detail in Chapter 42.

Discussed next are specific areas of dysfunction that present frequent challenges for the child with a brain tumor and that carry significant potential for affecting a child's quality of life.

### *Endocrinopathies*

Endocrine dysfunction may occur as an adverse effect of a brain tumor itself, as a consequence of increased intracranial pressure, or secondary to treatment. Hormonal disturbance is common with craniopharyngioma, and children may present with small stature and diabetes insipidus, due to hyposecretion of growth hormone and antidiuretic hormone, respectively. Endocrinopathies are among the most treatable long-term sequelae of radiotherapy. The radiation port for the cervical spine includes the thyroid gland, whereas the port for the posterior fossa includes areas involved with growth hormone–releasing hormone. In children who receive whole-brain irradiation, the entire neuraxis is irradiated, and these youngsters are at risk for a variety of endocrinopathies, including growth hormone deficiency, secondary and tertiary hypothyroidism, and cortisol deficiency. Growth failure is the most common sequela. In addition to growth hormone deficiency, growth failure may be due in part to failure of vertebral body growth in children with brain tumors who have undergone craniospinal irradiation. Because it affects cosmesis, short stature should be addressed when considering the needs of the child with cancer. It is of particular concern in adolescents and may adversely affect a youngster's psychosocial development. Exogenous hormone therapy may be given until epiphyseal fusion occurs and growth deceleration can no longer respond to trophic hormones.[25]

### *Sensory Deficits*

Sensory deficits may occur owing to direct involvement of the tumor or as a result of the antitumor therapy. Visual loss, visual field deficits, gaze palsies, and involuntary ocular movements all may damage vision and result in significant functional impairment. Visual disturbances are very common in craniopharyngiomas, owing to compression of the optic chiasm. Thorough ophthalmologic assessment should be completed and visual function monitored as part of the rehabilitation plan. This is particularly important in young children with oculomotor abnormalities who may require treatment to prevent amblyopia. Of note, focal pathology does not always signify focal disease. For example, the abducens nerve has a long, free intracranial course and passes in close proximity to bony structures. It may be compromised owing to elevation in intracranial pressure, with resultant sixth nerve palsy and diplopia. The occupational therapist can address compensatory strategies for visual deficits in addition to addressing visuomotor and visuoperceptual deficits. With severe vision impairment, a low-vision specialist should be part of the rehabilitation team. Adjunct service providers usually are available through the state agency for the blind, the school district, or special schools for the blind or visually impaired. If visual impairment is severe, referral should always be made to the state commission for the blind for adjunct services and equipment needs.

Hearing loss can result from tumor involvement or as a consequence of irradiation or chemotherapeutic agents such as cisplatin and carboplatin. Cisplatin produces high-frequency sensorineural hearing loss and tinnitus, the latter of which usually subsides. The hearing loss is almost always permanent and is primarily due to injury to the hair cells of the organ of Corti, although damage to the stria vascularis has also been described. Factors associated with a higher risk of cisplatin toxicity include prior or concomitant cranial irradiation, preexisting hearing loss, decreased renal function, concomitant use of other ototoxic drugs, faster infusion rate, higher peak plasma concentration, very young age or older age, and higher cumulative dose, in addition to individual susceptibility. [26] As regards carboplatin, studies have revealed high-frequency hearing loss but minor or no loss of hair cells. Baseline audiologic evaluation should be provided for all children, with regularly scheduled reevaluations according to the type of treatment provided. Hearing assessment should precede initiation of speech and language therapy services. Depending on the deficits, amplification may be warranted in the form of a hearing aid or auditory trainer. For the young child with severe hearing loss, instruction in sign language may be appropriate, in addition to training in oral language skills. For the child who has been exposed to chemotherapeutic agents that are ototoxic, care must be taken to

limit further exposure to ototoxic agents, even in the absence of hearing deficits, as the toxic effects may be cumulative.

Impairments of smell and taste are less recognized sensory deficits but can be seen with tumors that involve the region of the olfactory nerve. These deficits become significant when they affect appetite and, consequently, nutrition.

### *Cognitive Impairment*

At diagnosis, cognitive impairment is most common with hemispheric and supratentorial midline tumors. Deficits in memory, language acquisition and comprehension, attention, and academic skills vary with age at diagnosis, type and duration of presenting symptoms, tumor extent, and treatment. With improved survival, the impact of cranial irradiation on cognitive function and academic potential has gained increased significance. Radiotherapy is associated with a significant decline in cognitive function that is inversely related to age at diagnosis. A mean intelligence quotient (IQ) loss of 27 points has been demonstrated 2 years after cranial irradiation in children younger than 7 years, whereas at reevaluation no significant difference was seen in the performance of older children after radiotherapy. [27] Younger children, particularly those younger than 3 years, have been found to be more susceptible to the negative cognitive effects of radiotherapy, with reported drops in IQ of as much as 40 points. Deficits have included significant impairment in verbal and performance IQ, perceptual-motor skills, language development, and attention and executive skills in those children who received cranial irradiation as compared to nonirradiated children. [28]

Identifying and understanding factors other than radiotherapy that may contribute to neuropsychological impairment in survivors of brain tumors are essential for the rehabilitative team. A more accurate assessment of the neuropsychological status of a child with a brain tumor may be obtained by considering the accumulation of prediagnostic and postdiagnostic medical events resulting in brain injury, in addition to the treatment-related complications that affect cognition. [29]

When feasible, baseline neuropsychological assessment should be undertaken prior to or shortly after initiating treatment. When deficits exist, therapeutic and educational services should be instituted, if appropriate, based on the child's status. When no deficits are present, cognitive status should be monitored on a regular basis with neuropsychological reassessments. Children younger than 3 years should be referred to an early childhood intervention program for monitoring and stimulation of developmental progress, appropriate therapies, and parent education. An advantage of this type of program is that services can be provided in the child's home, which decreases risks of exposures to the common communicable diseases for the child who is immunosuppressed.

For the school-age child, close communication between the treatment team and the child's educational program is critical to ensure appropriate bidirectional flow of information regarding the child's level of function; academic, therapeutic, and psychoemotional status; and recommended services. The importance of providing an appropriate educational program is discussed in Chapter 50.

### *Communication Deficits*

In addition to deficits in speech and language previously discussed, cerebellar mutism syndrome may occur after posterior fossa surgery. One of the remarkable features of this poorly understood syndrome is the delayed onset. In the immediate postoperative period and for as long as 5 days afterward, a child may exhibit normal speech production. This is followed by a sudden cessation of speech, with preservation of symbolic functions and without evidence of impairment of cranial nerves or peripheral organs of speech. Rate of recovery varies from days to months. In most cases, during recovery, ataxic speech patterns—characterized by staccato, slurred, explosive, and irregular patterns and typically associated with cerebellar involvement—are absent. Instead, “ataxic dysarthria” and scanning speech has been described. [30] Some children are hoarse and hypernasal or exhibit a strained or strangled vocal quality. Children with cerebellar mutism have also demonstrated significant high-level linguistic and cognitive deficits on formal speech-language and neuropsychological testing. These deficits are consistent with those aspects of cognition associated in recent literature with the cerebellum (e.g., processing speed, memory, and cognitive planning). [30, 31]

### *Oral Motor Dysfunction*

With involvement of the lower cranial nerves and bulbar dysfunction that can occur with tumors of the posterior fossa, swallowing and deglutition dysfunction may occur. The speech-language pathologist is responsible for providing clinical evaluation of the swallowing mechanism and participates with the radiologist in videofluoroscopic evaluation, when warranted, to ensure that the child can safely be fed orally. Silent aspiration is a common finding with bulbar dysfunction, especially in the presence of pharyngeal sensory deficits.

## Spinal Cord Tumors

Spinal cord dysfunction occurs in up to 4% of children with systemic cancer. [32] Sarcomas account for the majority of epidural metastases, followed by neuroblastoma, lymphoma, and leukemia. Symptoms of metastatic cord compression include back pain, weakness, sphincter dysfunction, and sensory abnormalities. [32] The most common intramedullary tumors are astrocytoma and medulloblastoma. [33] The initial evaluation and medical management of these lesions are covered in Chapter 27. Children with a spinal cord injury (SCI) have a unique set of medical and rehabilitation issues.

A number of medical complications may affect a child's ability to participate in therapy after an SCI. Altered respiratory function, including diminished vital capacity and forced expiratory volume, may occur in the setting of lesions of the thoracic and cervical cord. Weak abdominal muscles allow the diaphragm to be pulled down by gravity, further compromising respiration in an upright position; this can be lessened with the use of an abdominal binder. The child's caregivers should be educated in assisted coughing techniques to help clear secretions.

Sympathetic outflow is interrupted in lesions higher than thoracic level 6. Unopposed vagal tone may result in bradycardia, and decreased systemic vascular resistance may result in postural hypotension. Autonomic dysreflexia is a massive reflex sympathetic discharge that follows a noxious stimulus below the level of the spinal cord lesion. Common causes include a distended bladder or stool impaction; however, any noxious stimulus below the level of the lesion should be considered. Symptoms include headache, flushing, sweating, decreased or increased heart rate, and hypertension. Autonomic dysreflexia can cause hemorrhage, seizures, and even death. If autonomic dysreflexia is suspected, the patient should be placed in an upright position, and a cause should be sought. Typically, when the noxious stimulus is removed, the blood pressure quickly returns to baseline.

Children with SCI are at risk for deep venous thrombosis. The exact incidence of deep venous thrombosis in pediatric SCI is unknown; one study reported an incidence of 10% in children ages 15 to 18 years and 5% in children younger than 15 years. [34] No standard recommendations for pediatric deep venous thrombosis prophylaxis have been devised. The Consortium for Spinal Cord Medicine has published guidelines for prophylaxis in adults. [35] Recommendations include the use of compression hose or pneumatic devices for all patients with SCI during the first 2 weeks. Anticoagulant prophylaxis with either low-molecular-weight heparin or adjusted-dose, unfractionated heparin should be started within 72 hours of injury if no contraindications exist. Anticoagulants should be continued until hospital discharge in incomplete injuries, for 8 weeks in patients with uncomplicated complete motor injury, and for 12 weeks for patients with complete injury and additional risk factors, such as cancer.

Immobilization results in increased urinary excretion of calcium, which may last many months, predisposing patients to urolithiasis. [36] Immobilization hypercalcemia presents typically in adolescent males 4 to 12 weeks after injury. Signs and symptoms such as lethargy, alteration of mood, nausea, anorexia, and polyuria are nonspecific; therefore, serum calcium levels should be periodically monitored. [36]

Spasticity often occurs as a consequence of an upper motor neuron lesion. It can cause pain or decreased range of motion or interfere with mobility and self-care. A syrinx should always be considered if one notes a sudden increase in spasticity that had been stable. Initial treatment should include alleviation of any noxious stimuli that may increase spasticity, stretching, and splinting. Oral baclofen should be considered if these measures do not alleviate the spasticity satisfactorily.

SCI commonly causes a neurogenic bladder. The management goals are adequate emptying, continence, and prevention of infection and upper tract damage. Spontaneous, uninhibited contractions of the detrusor occur with an upper motor neuron bladder. Detrusor-sphincter dyssynergia occurs when the external sphincter reflexively contracts simultaneously with the detrusor. This can result in a high-pressure bladder, placing the upper urinary tract at risk. Lower motor neuron lesions result in a flaccid external urethral sphincter and detrusor, predisposing to incontinence. Intermittent straight catheterization often is necessary to facilitate bladder emptying and increase continence. At 5 to 7 years of age, children with adequate hand function can begin self-catheterization. Fluid intake should be regulated so that only four to five catheterizations are necessary without exceeding the bladder capacity [age (years) + 2 = ounces]. [37]

SCI also frequently results in a neurogenic bowel. Upper motor neuron lesions result in spastic contraction of the external sphincter that can lead to incomplete emptying. Adequate fluid and fiber intake is necessary for a successful bowel program. Often, digital stimulation, suppositories, stool softeners, and bulking agents also are necessary to ensure adequate bowel emptying. Lower motor neuron lesions result in a flaccid sphincter, predisposing to incontinence that is best managed with a regular emptying program.

Rehabilitation efforts are aimed at maximizing muscle strength and range of motion and facilitating independence in activities of daily living and mobility. Greater independence can be expected in individuals with lower levels of SCI and with incomplete injuries. An adult with SCI at a level as high as C7 can live a completely independent life.

Children with tetraplegia are prone to deformities of the upper extremities, particularly elbow flexion, forearm supination, and metacarpophalangeal extension contractures.[38] Contracture scan preclude the development of tenodesis (passive grasp with wrist extension) and successful reconstructive tendon transfer procedures. During acute rehabilitation, children should work with occupational therapists and nurses on self-care skills. Orthotic devices such as universal cuffs, balanced forearm orthoses, and wrist-driven flexor hinge orthoses may increase a child's level of independence. A number of factors influence a child's use of orthoses, including the size and weight of the orthosis, the child's understanding of the purpose of the orthosis, parental support, and independent ability to use the device at school.[38] A wide variety of adaptive devices are available that may be useful, such as built-up utensil handles, scoop dishes, sock loops, button hooks, adapted mirrors, and long-handled sponges.

Many aspects of mobility must be addressed even if ambulation is not a goal. Therapists should address with the patient such skills as turning in bed; assuming a sitting position; sitting balance; transfers between wheelchair, bed, toilet, and car; wheelchair skills (including “wheelies”); and driving, if age-appropriate. Ambulation is possible for children with paraplegia. Children with lesions at levels T11 to L2 can be functional indoor ambulators with the help of long leg braces and an assistive device. In children with lesions above T11, ambulation is very slow and is best viewed as exercise. [36]

The family and medical team should be familiar with the child's capabilities so that the child's independence across settings within the community can be maximized. Community reentry activities are essential if the child is to become familiar with negotiating common architectural barriers such as curbs, heavy doors, and inaccessible areas; such activities will aid the child in solving problems related to negotiating barriers or will help him or her to learn to ask for assistance. Discharge planning should address independence in the child's home, school, and community, including recreational activities.

## Bone Tumors

Osteogenic sarcoma (osteosarcoma) is the most common malignant bone tumor of childhood, followed in frequency by Ewing's sarcoma.[39] Until recently, amputation was the usual treatment for these tumors of the extremities in children.[40] Improved survival has followed advances in surgical techniques and the use of adjuvant treatments, as discussed in Chapter 33 and Chapter 35. Five-year survival rates for patients with these tumors, if localized, now exceed 60%. [39] Currently, several surgical options to amputation exist, and the rehabilitative team's involvement currently includes, but is no longer limited to, provision of external prostheses. [40] Prior planning of the rehabilitation program for children affected with bone tumors is recommended for any of the surgical options. [39]

The various approaches to limb-sparing surgery include use of allografts or autografts, rotationplasty, and endoprosthetic reconstruction. [40] Future growth, functional demands, individual preference, and life expectancy should all be considered. [39]

### *Rotationplasty*

Van Nes rotationplasty, usually performed for lesions involving the distal femur since its application to malignant tumor surgery in 1981, converts an above-knee amputation to a functional below-knee amputation. After the tumor is resected, the proximal tibia is rotated 180 degrees and fixed with an intramedullary rod to the stump of the femur. The desired length of the new thigh is calculated to be as nearly equal in length to the contralateral thigh at skeletal maturity as is possible. The ankle is aligned with the contralateral knee, with the plantar aspect of the foot now facing forward ( Fig. 45-3). The foot functions as a tibial stump in a below-knee amputation, and the ankle joint functions as a knee joint (Fig. 45-4). The relative advantages of this technique over the endoprosthetic options (discussed below) are the relatively low rate of complications, excellent functional outcomes (such that many children are able to participate in sports at a level approaching the activity level of a child with a below-knee amputation), and accommodation for future growth of the extremities.[41] It is recommended as an alternative to endoprosthetic replacement for skeletally immature individuals, particularly for those who place function ahead of cosmesis. [41] Some surgeons who advocate strongly for consideration of patient age in the procedure selection process recommend Van Nes rotationplasty for children who are younger than 10 years. [42] Frequently mentioned disadvantages of the rotationplasty are cosmesis and potential adverse psychological impact. [41] However, assessment at least 1 year after rotationplasty in adults and older teenagers reveals levels of psychosocial functioning, general quality of life, and social support that are highly comparable to those of healthy peers. [43]

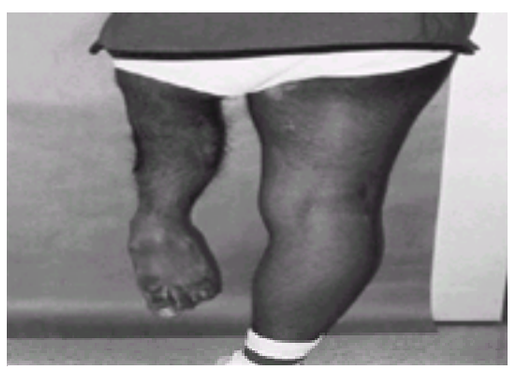

**FIGURE 45-3.** After resection of most of the femur, Van Nes rotationplasty allows ankle dorsiflexors to function as knee extensors and plantar flexors to function as knee flexors. Note alignment of the ankle with the contralateral knee after rotationplasty. Rotationplasty in this patient converted what would otherwise have been a hip disarticulation level of amputation to a functional below-knee level for purposes of prosthetic fitting. (See Figure 35-13.)

**FIGURE 45-4.** The Van Nes prosthesis, with the heel situated in the socket at the level of the contralateral patella, may not be obvious under clothing. It can result in good function as well as cosmesis, especially relative to the option of a hip disarticulation prosthesis. (See Figure 35-13.)

### *Endoprosthetic Reconstruction*

Limb salvage therapy, combining wide tumor resection with endoprosthetic replacement and adjuvant chemotherapy, has become a popular option to amputation or rotationplasty for primary bone sarcoma (osteosarcoma, chondrosarcoma, malignant fibrous histiocytoma). [40,44] The consensus of the Committee of Pediatric Orthopaedics of the American Academy of Orthopaedic Surgeons is that limb salvage surgery is preferable to amputation when survival is not compromised and that upper limb salvage is more important than lower limb salvage.[39] Endoprosthetic reconstructions give satisfying cosmetic and functional results in most patients. [40,44] Patients with distal femoral endoprosthetic reconstruction (Fig. 45-5 and Fig. 45-6) achieve the highest functional outcomes, whereas patients with total or push-through femoral replacements achieve the lowest functional outcomes.[44] During the first 6 months postoperatively, 80% of patients in one study, two-thirds of whom had undergone reconstruction with modular prosthetic arthroplasties, were unable to walk without support. [45] Function generally improves progressively throughout the second 6-month period and the first and second years after amputation, rotationplasty, or limb salvage procedures. [45] Functional outcomes can be expected to be as good with a revised as with a primary endoprosthesis.[44] Functional results after limb-sparing surgery are better for the upper than the lower extremity. [46]

**FIGURE 45-5.** Endoprosthesis to replace resected distal femur and knee joint. (Device manufactured by Stryker Howmedica Osteonics, Allendale, NJ. Photo courtesy of Howmedica Osteonics.)

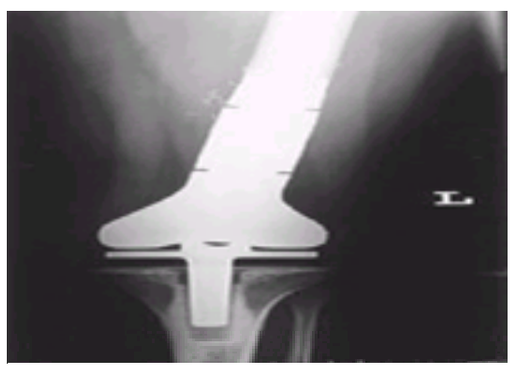

**FIGURE 45-6.** Anteroposterior radiograph of a 16-year-old girl 6 months after wide resection of a distal femoral osteosarcoma and reconstruction with the endoprosthesis pictured in Figure 45-5. (Courtesy of Dr. John P. Dormans, Chief, Division of Orthopaedic Surgery, Children's Hospital of Philadelphia.)

### *Physical Rehabilitation after Limb-Sparing Procedures*

Physical rehabilitation after limb-sparing procedures is more difficult than that after amputation. [47] Early and more aggressive rehabilitation programs result in better outcomes.[44] Specific regimens vary with the surgical site, procedure, and surgeon. Exercise regimens can often start 1 to 2 days postoperatively. [40,44] Range-of-motion exercise provided by a continuous passive motion machine can also be initiated after distal femoral reconstruction either in the recovery room [48] or 1 to 2 days postoperatively, with active range-of-motion exercise started subsequently.[44] After proximal tibial replacement, a less aggressive approach may be indicated; gentle range-of-motion exercises may not be undertaken until after a 2- to 3-week period of casting in full extension. [44,48] Standing may be appropriate within 1 week after endoprosthetic replacement surgery, unless a muscle flap procedure was performed.[40] Gradual weight bearing using two crutches may be possible 2 weeks postoperatively, with progression to full weight bearing after a few months, [44] although ambulation with the use of a knee immobilizer is begun by some a few days after distal femoral reconstruction.[48] Bracing at the knee can augment stability after procedures involving the femur or tibia. [40] Continuous passive motion machines can be used at home after discharge for another 1 to 2 months.[48] For patients with total or proximal femur replacements, bed rest with hip abduction for 2 to 4 weeks, followed by hip abduction bracing for 3 months, may be recommended.[48] Upper extremity endoprosthetic reconstruction can be followed by use of shoulder immobilization for 2 to 3 weeks before physical therapy is begun, to maximize shoulder and elbow range of motion. [48] These regimens result in fewer difficulties in establishing extremity function than did earlier methods in which primary wound healing was achieved prior to initiating assisted active exercise. [44]

In skeletally immature patients, allowance must be made for future growth after endoprosthetic implantation. Efforts are under way to use an electromagnet to extend modular endoprosthetic systems noninvasively.[49] Currently, intercalary segments are surgically exchanged to equalize extremity length during the period of growth. [48] Different systems are appropriate for very young (5 to 8 years) and older children, an adjustable and expandable prosthesis being recommended by some surgeons for the youngest patients and modular systems being suggested for large preadolescents and adolescents. [48] Expectation that the family will participate in the rehabilitation and follow-up efforts is advisable before embarking on an expandable endoprosthetic reconstruction in the skeletally immature patient. [48]

Most endoprosthesis-related complications are mechanical failures and may require extensive revisional surgery. [44] Such complications or the need for revision occurs in 41% to 56% of cases, as reported in recent series. [42,44,45,48] Infection after endoprosthetic reconstruction may necessitate amputation.[44] To date, “durable reconstruction is elusive.”[42] Amputation or rotationplasty procedures have approximately one-third the complication rate of endoprosthetic reconstructions. [45]

Almost half of all children who undergo resection of primary bone tumors with an expandable endoprosthetic replacement will not require use of an orthosis or gait aid.[40] Unassisted ambulation is more likely if the quadriceps mechanism is preserved, either after expandable endoprosthetic replacement [40] or after prosthetic knee replacement following distal femur bone tumor resection.[42] More extensive loss of the active quadriceps mechanism with endoprosthetic replacement at the level of the proximal tibia results in loss of active knee extension and knee instability and a worse functional outcome than is seen with distal femur replacement. [40] The rehabilitative needs of children who undergo limb-sparing surgery include early mobilization, gait training, and continued follow-up to monitor activity restriction. [40] Shoe lifts can be used on the contralateral limb to address the leg length discrepancy between lengthening procedures. [40]

Some functional limitations can be expected after endoprosthetic reconstruction. Patients may be advised after lower extremity endoprosthetic reconstruction to use a cane out-of-doors permanently and not to participate in sports other than swimming.[44] Although children with endoprostheses are restricted from high-speed, high-impact sports and activities requiring a high degree of coordination—specifically football, tennis, soccer, and field hockey—using a stationary bicycle, walking, hiking with a cane, swimming, and participating in a modified program of physical education at school are permitted. [40]

Children with expandable endoprostheses may face issues related to adjustment to the need for repeated hospitalizations for lengthening. [40] Several studies of children and adolescents with acquired limb loss show remarkably good psychosocial adjustment in this population, regardless of surgical approach. [47] No significant

differences are in evidence in quality of life or global physical and psychological functioning as assessed 1 to 3 years postoperatively in children and adolescents managed with limb-sparing procedures plus adjuvant therapy as compared with those managed with amputations and adjuvant chemotherapy.[47]

Although currently a child or adolescent with a primary bone tumor is more likely to be offered a limb-salvage operation and reconstruction than an amputation, which type of surgical intervention results in a superior functional result remains unclear. [50] A single recent comparison study of patients treated for pediatric malignant bone tumors with either amputation or limb-sparing surgery showed no significant differences at a median of 14 years postoperatively in functional limitations, educational or occupational status, pain, self-image, interpersonal interactions, or overall satisfaction with their surgical procedure. [46] In each group inthis study, approximately 90% of patients reported limitations in running and lifting heavy objects, 75% to 90% reported limitations in contact or team sports, and 50% reported limitations in recreational activities and bending, kneeling, and stooping.

***Future Developments***

Use of a recently developed system for functional evaluation of surgical procedures for musculoskeletal tumors has been adopted by the Musculoskeletal Tumor Society, to allow future comparisons across study populations.[50] The tool is based on analysis of those factors pertinent to the patient with a musculoskeletal tumor (pain, functional activity, emotional acceptance) and those factors specific to either the upper limb (positioning of the hand, manual dexterity, lifting ability) or the lower limb (use of external support, walking ability, gait).[50] As surgical techniques and postoperative management continue to evolve and more children and adolescents survive longer, such a measure ought to be useful in comparing future functional outcomes.

**Leukemia**

Leukemia is the most common malignancy in childhood. Acute lymphoblastic leukemia (ALL) accounts for approximately 75% of all cases of leukemia, and the current cure rate for ALL is 70%.[51] Because the majority of children survive into adulthood, the rehabilitation team must address not only the effects of the disease itself but also the long-term effects of treatment. In addition, the team must be aware that leukemia has the potential for a course of remissions and exacerbations, and so rehabilitation issues are likely to change over time.

Bone pain caused by proliferation of hematopoietic tissue within the medullary cavity is a frequent complaint in acute leukemia. The pain most commonly occurs in the lower extremities and usually is intermittent, well-localized, sharp, severe, and sudden in onset. [52] Radiographic skeletal changes may include osteopenia, radiolucent metaphyseal bands, lytic lesions, sclerotic lesions, or pathologic fractures. [53] No widely accepted method exists for determining the risk of pathologic fracture in involved bone. The risk may be higher with painful lytic lesions and in other cancers with metastases where the ratio between the width of the metastasis and bone is greater than 0.6 or if there is cortical destruction of the circumference greater than or equal to 50%. [54] Attempts should be made to reduce weight bearing through areas at risk, and resistive strengthening activities should be avoided. Isometric strengthening and aerobic exercise such as swimming or riding a stationary bike should be considered. With spinal compression fractures, flexion activities of the spine should be avoided, and a corset or custom-molded spinal orthosis should be considered if significant pain is present. Aseptic necrosis should be considered in patients with hip pain who are taking corticosteroids. Corticosteroids may also contribute to osteopenia. Osteopenia tends to improve gradually after disease remission, whereas bone pain tends to improve rapidly after chemotherapy or radiotherapy.[52]

A peripheral neuropathy or myopathy should be considered in patients with progressive weakness. Myopathy is a common complication of corticosteroid therapy and a rare complication of vincristine therapy. It classically presents with the insidious onset of painless, symmetric, proximal muscle weakness that leads to difficulties in arising from a low chair, climbing stairs, and performing overhead activities. The electrodiagnostic examination may reveal few abnormalities, owing to the preferential atrophy of type II fibers, which are not evaluated by electromyography. [55] The myopathy usually is reversible if the drug is withdrawn or the dose reduced. [56] The rehabilitation program should include passive stretching and proper positioning of the hip, knee, and shoulder, with particular focus on the hip flexors, hamstrings, iliotibial bands, and shoulder adductors and internal rotators. Strengthening and endurance exercise can lessen but not eliminate glucocorticoid-induced muscle atrophy and weakness.[57]

Vincristine therapy commonly causes an axonal, sensorimotor polyneuropathy. Loss of ankle jerks and complaints of numbness and tingling in the feet or hands usually precede the onset of distal weakness. The weakness may progress to involve the more proximal limbs but generally recovers rapidly if the drug is stopped or the dose reduced.[56] Therapy should focus on passive stretching of the wrist and finger flexors as well as the gastrocnemius-soleus complex. If the weakness is severe, a resting wrist-hand splint may be worn at night and periodically during the day to maintain range of motion. A splint may also be used at the ankle to maintain at least a neutral position (zero degrees) in dorsiflexion. If the weakness causes footdrop during ambulation, a custom-molded ankle-foot orthosis should be considered.

Hematologic abnormalities may also affect a patient's mobility and ability to perform exercise. No standard hematologic parameters for exercise have been devised. Low platelet counts increase the risk for cerebral, intramuscular, and joint hemorrhage during exercise. In a study of patients with ALL, visible hemorrhage was rare with platelet counts greater than 20,000, and no intracranial hemorrhage occurred with platelet counts greater than 10,000. [58] In general, moderately vigorous exercise can be pursued when platelet counts are at least 30,000 to 50,000 and low-impact aerobics, but not resistive activities, can be considered with counts in excess of 10,000 to 20,000.[59] Exercise is not recommended with platelet counts of fewer than 10,000.[60] It has also been suggested that exercise be discontinued with a hemoglobin of less than 7.5 g and a white blood cell count of fewer than 3,000. [59]

Anthracyclines and cardiac radiation have the potential to cause acute and long-term cardiotoxicity, including ventricular dysfunction, pericarditis, electrocardiographic abnormalities, and arrhythmias.[61,62] The risk for cardiac abnormalities is dose-dependent. Potentially serious ventricular ectopy has been noted in patients with cumulative doses exceeding 200 mg per square meter.[62] Evaluation of patients who have received anthracyclines or heart irradiation should include an echocardiographic shortening fraction, a resting electrocardiogram for evaluation of QTc interval, and a history of exercise intolerance. [63] A 24-hour Holter monitor also is recommended, because Holter results do not correlate with resting studies. [63] A pediatric cardiologist can help to provide safe exercise precautions for patients with cardiac abnormalities.

The long-term impact of weakness and deconditioning on gross motor function related to ALL and its treatment has not been fully investigated. One study of 36 children treated for ALL (median time off therapy, 40 months) revealed significantly decreased strength, balance, running speed, and agility on the Bruininks-Oseretsky Test of Motor Proficiency, as compared to 36 age- and gender-matched controls. No statistical differences were found on basic motor skills such as standing, running, and jumping using the Gross Motor Function Measure.[64] A tendency for obesity has been noted in survivors of childhood leukemia; weight-for-age data did not reveal a correlation with motor skills. [65] The long-term gross motor abnormalities in ALL appear to be subtle and may go unnoticed, yet competence in gross motor abilities contributes to a child's self-esteem, level of fitness, and success in and enjoyment of recreational activities. [64]

Long-term cognitive deficits have been associated with irradiation and intrathecal methotrexate therapy used for CNS prophylaxis. There are no good epidemiologic estimates of the prevalence of cognitive deficits related to the variety of factors that may influence cognitive outcomes, including age and gender at diagnosis and the specific protocol used. Likewise, no widely accepted outcome measures are available. A number of studies have evaluated the child's IQ, but IQ is not sensitive to the subtler aspects of information processing that can result in learning and social difficulties. [66] Survivors of ALL may have difficulty in a wide variety of areas, including problem solving, organizing information, memory, inferential reasoning, and social interaction. [66] Children may do well in early grades but experience increasing difficulty in higher grades, when greater efficiency and the ability to work more independently are required. Parents should be alerted to the possibility of difficulty presenting in higher grades, and any indication of academic difficulties should be pursued with a professional evaluation.

## BONE MARROW TRANSPLANTATION

Bone marrow transplantation (BMT) is being used for an increasing number of life-threatening disorders. BMT patients are at risk for significant morbidity, related to the high-dose chemotherapy or total-body irradiation (or both) during the preparatory regimen and immunosuppression after the BMT. Fatigue, weakness, and pain are common and can impose limitations in the child's ability to perform self-care skills as well as in educational and leisure activities. Participation in a rehabilitation program that includes aerobic exercise can significantly increase a patient's physical capacity without increasing morbidity. [67] Suggested rehabilitation goals after BMT include (a) reduction or prevention of muscle atrophy from disuse; (b) prevention of pneumonia and promotion of good pulmonary circulation; (c) maintenance of joint range of motion; (d) maintenance of balance, coordination, and endurance; (e) prevention or treatment of depression; and (f) promotion of physical and emotional

well-being.[60]

The rehabilitation team should be aware of a number of medical complications that may require modification of the patient's therapy. All patients after BMT undergo a period of bone marrow suppression. The thrombocytopenia places the patient at risk for hemorrhage, and leukopenia can lead to life-threatening infections. Fever may adversely affect a patient's exercise tolerance, and therapy usually is withheld during the presence of a fever higher than 40 $^{o}$C.[68] The patient's skin may be vulnerable, owing to irradiation or graft-versus-host disease, and protection should be provided against excessive stress, force, massage, or heat. [68] Patients with veno-occlusive disease or graft-versus-host disease associated with abdominal distention and pain should avoid activities that strain their abdominal muscles. Respiratory complications, including pneumonitis, are common, and so the patient's respiratory status should be closely monitored. Medications should be reviewed for potential side effects, such as the potential for cardiomyopathy associated with cyclophosphamide.

Psychological factors should also be taken into consideration. Transient depression, anger, and withdrawal are commonly encountered and may lead to poor cooperation and therapy refusal. [60] Gentle encouragement and “bargaining” are often helpful in eliciting a child's participation. Allowing a child the ability to select his or her appointment time or sequence of therapy may increase the child's sense of control and thus increase participation. A successful rehabilitation program requires both flexibility and persistence on the part of the rehabilitation team, owing to the frequent number of medical complications, the psychological adjustment of the patient, and the unpredictability of the patient's course of recovery on any particular day.

## CRITICAL-ILLNESS POLYNEUROPATHY AND MYOPATHY

Neuropathy and myopathy are common complications of critical illnesses. [57,69] The incidence of peripheral nerve abnormalities in patients with sepsis and multiple organ failure can be as high as 70%, with clinical signs of critical-illness peripheral neuropathy occurring in approximately one-half of these patients. [70] The etiology is believed to be related to a disruption of blood flow to the distal sensory and motor axons owing to a systemic inflammatory response. [71] The first sign of critical-illness neuropathy often is difficulty weaning from the ventilator, after the exclusion of pulmonary and cardiac causes. Weakness typically starts symmetrically in the distal limbs and may progress proximally. Sensory loss and reduced muscle stretch reflexes are common.[57] Recovery depends on the severity of nerve involvement. In mild cases, recovery can occur over weeks. Severe cases may take months to resolve and, in some cases, deficits are permanent.[70]

Acute necrotizing myopathy and acute myosin filament loss myopathy have been associated with critical illness. [57] Acute necrotizing myopathy typically is seen in patients on prolonged courses of neuromuscular blocking agents and high-dose corticosteroids. It is characterized by generalized weakness, areflexia, normal sensation, occasional ophthalmoplegia, and a markedly elevated creatine phosphokinase level. [72] Proximal weakness and respiratory dysfunction characterize acute myosin filament myopathy. Sensation typically is normal, and the creatine phosphokinase level is normal or only transiently elevated. This myopathy generally is observed in patients who are taking high doses of corticosteroids, with or without concomitant use of neuromuscular blocking agents. [72] In general, the prognosis is favorable, although children with severe muscle involvement are more likely to experience prolonged or incomplete recovery. [57]

The etiology of acute weakness associated with critical illness is not always readily apparent; some authors have suggested that critical-illness neuropathy and myopathy are not as clinically distinct as is often described. [57] The possibility also exists for more than one entity to be present simultaneously. A history of the hospital course, including medications, a thorough neurologic examination, and electrodiagnostic testing will help to determine the most likely etiology and aid in the prognosis for return of function.

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# PSYCHIATRIC AND PSYCHOSOCIAL SUPPORT FOR THE CHILD AND FAMILY

STEPHEN P. HERSH
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## INTRODUCTION

Since the 1960s, advances in treatment techniques, as well as supportive care, have resulted in dramatic improvements in the survival rates of children with cancer. Today, the focus of pediatric oncology includes a heightened concern about the development and quality of life of the child, siblings, and parents. The child's adaptation, both during treatment and as a long-term survivor, is of paramount concern. For the child with cancer and the child's family, successful developmental and psychosocial outcome hinges on thoughtful assessment, a dedication to prevention, and early intervention orchestrated by the treatment team.

This chapter addresses the impact of childhood cancer on both the child and family. We survey the phases of illness from diagnosis through the stages of treatment and the issues faced, such as reentry into the community and the uncertainties of long-term survival. Vulnerabilities, points of stress, and potential disruptions are discussed. Developmental implications for child and family as well as appropriate educational, psychological, and psychiatric support measures are presented. We recommend interventions and strategies aimed at building on child and family strengths, providing support, handling stresses, and enhancing adaptive coping skills.

## PRINCIPLES AND ESSENTIAL KNOWLEDGE FOR HEALTH PROFESSIONALS

The diagnosis of cancer has a powerful and lasting impact on child, family, and their immediate community. Families must endure the transition from feeling in control of their lives to living with constant uncertainty. The need to depend on the medical system for answers and cure requires enormous adjustment by the parents. No family member—sibling, grandparent, or other relative—is unaffected.

Most children with cancer survive for extended periods. Many children, when offered early diagnosis combined with state-of-the-art treatments, are cured. Even with cure, the impact on the family persists. Marriages, careers, and relationships with others are significantly affected. Some families become more cohesive, developing increased strengths and a positive redefinition of values. [1,2] Others, often those with preexisting vulnerabilities, suffer various degrees of chronic disequilibrium.

Understanding the challenge that cancer and its treatment present to both child and family requires an awareness of our culture, its beliefs, its fears, its pluralism, and its problems. The fears associated with cancer include loss of control, fear of death, fear of disfigurement, images of punishment for unnamed transgressions, and guilt.[3] These fears combined with the extraordinary family destabilizing social changes that have occurred over the past 30 years create a situation in which cancer severely tests the strength of any family system. Destabilizing social changes include (a) movement away from the influence of traditions structuring daily life; (b) the powerful, intrusive influence of the media (magazines, movies, newspapers, radio, television) and the Internet on expectations, ideas, and information; (c) the dramatic increase in the number of families with both parents working outside the home; (d) increases in divorce and remarriage rates [4]; (e) the dramatic growth of single-parent families[5]; (f) increased reliance on various forms of day care; (g) increased delegation by parents to the school system of the responsibilities to train children in behaviors and values; (h) the progressive increase in the number of years spent in school rather than at work [6]; (i) the gradual reduction in family size and in the time members spend together[6]; and (j) high rates of substance abuse, suicide, and arrests for juvenile delinquency. These changes coexist with the harsh reality that families who have a child or adolescent with cancer may have other medical, psychological, or economic problems. And children who have cancer may not only be a member of a multiproblem family but may also have other physical or emotional illnesses.

The developmental experiences of every human being influence perceptions, understandings, and behaviors during life crises. A person's capacity to function, cope with stress, and survive evolves in interaction with life experiences. That evolution rests on a foundation of metabolic, motor, emotional, language, cognitive, and social capacities. Many of these may be genetically influenced. All are tempered in the context of accumulated life experiences.

Norms of behavior exist within each culture. Medical personnel must always be aware that behaviors vary in patients with loss of integrity of the central nervous system (CNS), poor nutrition, abnormal physiology, or significantly distorting life experiences such as abandonment, abuse, chronic unemployment, alcoholism or other substance abuse, or violence in the community.

People of all ages share the same complex range of responses to stress. At the neurophysiologic level, neocortical perceptions (such as being given the information that one's child has cancer) influence subcortical activity and other CNS functions, including the pituitary-adrenal axis, blood flow, vascular permeability, smooth muscle activity, immune system functioning, respiration, and temperature. Simultaneously, general physiologic, peripheral nerve, and organ states affect the CNS at its various levels. Psychologic concomitants of these responses coexist with thoughts and are recognized by the person as fear, guilt, tension, anxiety, “foggy-headedness,” anger, rage, hopelessness, or depression. Behavioral responses follow. No matter what form they take, these responses fall into the categories of alerting, fight, or flight.

All the above-mentioned interactions underlie the functioning and behaviors of the child, the parents, other family members—indeed the entire family system. Those

behaviors, in turn, affect the perceptions and behaviors of each individual person. A family's belief systems, history, and material resources influence its level of trust and experiences with control over the realities it faces. These, in turn, influence the family's sense of itself as intact, well-functioning, and strong, or, as fragmented, troubled, and insecure. Past experiences influence a family's capacity to adapt and to tolerate the many forms of stress. Past experiences that have shaken the family's confidence in having control over events make the family more vulnerable to dysfunction in the face of any new stresses such as a diagnosis of cancer.

The family forms a crucible within which not only children are molded but also all members are continuously “reworked.” Behaviors, fears, and values are learned within the family. The behavior and beliefs of family members powerfully influence the ways in which children and adults respond to new situations and to experiences such as discomfort, separation, loss of function, fear, guilt, and pain. Children affect parents by their reactions, just as anxiety, fears, feelings of guilt, and loss of control in parents and grandparents are all transmitted to children.

Families are best understood in terms of overlapping systems of behaviors, needs, perceptions, values, and personal resources (cognitive-experiential and spiritual as well as material). While observing interactions within a family, see them in terms of the interactions between parents and children as well as in terms of interactions among the various other possible combinations of relationships. (Examples include interparent, parents with an only child, both parents with each individual child, child and siblings, children and grandparents, grandparents and parents.) Power balances and shifts occur during times of crisis. Expectations shift. The family reevaluates its organizing principles and beliefs. Universally, families have certain experiences that trigger or stimulate their attention, produce an alerting response, and signal a potential loss of control. Such signals range from an obvious change in health status, such as a diagnosis of cancer, physical changes (e.g., cushingoid), or disfigurement (e.g., amputation), to more subtle changes, such as reduced activity level, pain, or changes in appetite or eating habits.

Health care professionals have come to recognize the complexity of delivering care to the child and family facing cancer. No single health professional can meet a family's needs completely. Through collaborative, multidisciplinary efforts, a health care team can provide comprehensive care that supports the entire family through the disease course (see Chapter 44, Chapter 45, and Chapter 50). The team can anticipate the psychologic adjustment of families and can plan appropriate interventions.

An essential component of this approach includes an assessment of the family's strengths and vulnerabilities ( Table 46-1). This information can be gathered and synthesized during one to three intake meetings, each lasting 45 to 60 minutes. The treatment team member who has training in child development and mental health should conduct these meetings. The setting should be as relaxed and unthreatening as possible. Intake is the optimal time for identification of families who are at high risk for the development of significant psychosocial problems over the course of the child's illness. Examples of preexisting psychosocial problems include (a) growth and development lags; (b) clinical psychiatric conditions (e.g., anxiety disorders, depression, patterns of impulsive behaviors and poor judgment, tic disorders, sleep cycle disruptions, pervasive developmental disorder, the schizophrenias); (c) consistent impairment in school or work performance; and (d) family system dysfunction (e.g., abuse; lack of communication; isolation from the community; noncompliance or overt sabotage of medical care). Families at high risk for these problems tend to share certain characteristics (Table 46-2). Children at high risk are described in Table 46-3.

**TABLE 46-1. SUGGESTED AREAS OF INQUIRY FOR OUTLINING THE FAMILY'S STRENGTHS AND VULNERABILITIES**

| |
|---|
| Single parents or two-parent families functioning as a single-parent family (e.g., spouse travels frequently, is a “workaholic,” or works more than one job or shift) |
| Preexisting chronic health or mental health problems |
| Parent incapacitated by health or mental health problem or substance abuse |
| Economic problems: rural or urban poor; overextended (debts) middle-class family; job loss and minimal or no health insurance |
| Separation; divorce |
| Stepfamily system |
| Chronic (unresolved) conflicts; parent–parent; parent–patient; parent–other children; sibling–sibling; grandparents–parents; grandparent–child |
| Language differences: immigrant; foreign national; significantly different subculture |
| Families away from their cultural support network because of the child's need for medical treatment |

**TABLE 46-2. FACTORS THAT PLACE FAMILIES AT HIGH RISK**

| Adaptive responses | Early signs of problems in coping | Maladaptive responses |
|---|---|---|
| Understanding by parents and other family members of diagnosis and prognosis | Forgetting or intermittently denying diagnosis or its implications and prognosis (i.e., cannot remember disease or treatments) | Unquestioning acceptance and unreserved compliance, or massive denial or refusal to accept diagnosis and explanations |
| Ability to seek information from medical staff, ask questions, take notes, seek second and third opinions | Inability to ask questions, or frantic activity, repeatedly asking the same questions | “Shopping” (seeking more than a third opinion) for a better, more acceptable diagnosis while avoiding potentially curative treatments |
| Ability to engage in necessary and appropriate explanations to patient and siblings; ability to make changes in lifestyle and life rhythms to accommodate the demands of diagnostic processes and treatments | Passive, slowly reactive | Inability of parents to perform ordinary tasks on behalf of patient, siblings, themselves, or family system |
| | Too busy to talk or “think about” feelings; partial withdrawal from one another within family (veil of silence); no expression to health care professionals | Only rudimentary communication among family members; increasing self-blame, guilt, and depression; use of “splitting” defenses, as in excessive criticism of some staff while aggrandizing others; assaultive anger directed at physicians, nurses, and the “system” |
| Ability to express feelings within family and with appropriate health care professionals (physician, primary nurse, social worker, hospital clergy) | | |

**TABLE 46-3. SPECTRUM OF ADAPTATION DURING DIAGNOSTIC PERIOD**

Intake is the best time to begin the never-ending process of educating the child and family to the illness, treatment system, and treatments. Ideally, one can set the stage for child and parents to become willing members of the treatment team rather than passive victims receiving care. Over the course of the many meetings during the intake period, the older child and the child's parents can be informed about special skills that they can learn (through biofeedback, hypnosis, relaxation training) to help in dealing with medical procedures and side effects. They can also receive help in learning how to work with doctors and ways to maintain social functioning, school attendance, appropriate limit setting, family chores, and work during the course of treatments.

## INITIAL DIAGNOSTIC PERIOD: A TIME OF CRISIS

### Family's Reaction to Diagnosis

How the diagnosis of cancer is presented to parents and their child not only significantly influences initial responses to medical interventions but also sets the attitudes that affect collaboration, compliance, and trust over the course of the illness. [3,7,8] Sophisticated oncologists develop an empathetic but direct style of disclosing a cancer diagnosis, a style tailored to the characteristics (subculture, education, language skills) and needs of each family, patient, and situation. The need to repeat information cannot be overemphasized.

> “It just doesn't feel real. I can't believe this is happening.”
>
> “I feel numb. I'm doing what I need to do but it doesn't feel like enough.”
>
> “I just don't understand how this happened. A kid doesn't just get cancer. There had to be a way that this could have been prevented.”
>
> “I'm scared. There are so many things to think about. She could die. . . . She really could die. And yet, here I am thinking about what this could do to my marriage.”

These comments were made at a support group for parents whose children had recently been diagnosed with malignancy. Parents see themselves as providers for their children, whom they are supposed to protect from fear, hurt, and pain. [9] The diagnosis of cancer represents an assault on a parent's identity and their sense of adequacy as guardian.[1] Shock, disbelief, guilt, anger, and fear are the usual emotional reactions experienced by parents after their child's diagnosis. [10] In response to these emotions, most parents struggle toward understanding the diagnosis and recommended treatments. Driven by a wish to reverse the implications of the diagnosis, families may seek second or more opinions as well as spend long hours talking to friends, visiting medical libraries, and accessing the Internet. Parents may selectively talk only to those acquaintances or relatives who promote unrealistic hopes or who encourage disbelief in what the doctors have told them. Initial disbelief allows the reality to be approached and integrated at a pace that does not overwhelm defenses. [11] Disbelief at first is protective. It reduces what could otherwise be intolerable anxiety, guilt, and anger.

As the diagnosis is accepted, guilt and anger become significant emotions. They may be directed in many ways. Anger, in particular, may be directed at physicians, other staff members, or the hospital at large. Those who were involved in the initial presentation of the diagnosis (a presentation that radically alters the family's life) may, for a short time, be the focus of significant negative feelings. Guilt is expressed in ruminations as parents seek reasons that cancer has occurred in their child. Many parents pass through a period of self-blame during which they focus on transgressions they may have committed and for which they feel they are now being punished. They may begin searching for evidence that they failed to pay sufficient attention to early signs of less than optimal health in their child. Some parents may berate themselves for not taking complaints seriously enough, for having children when “cancer runs in the family,” for smoking during pregnancy, or for living in polluted urban or industrial areas.

Careful listening (in the context of awareness of the family's cultural and linguistic background) and supportive attention by the oncology staff are important to these families. These families need reassurance (even if they verbalize no direct expressions of guilt) that they are not responsible for causing the disease. [12,13] Such interventions free parental energies for the extensive emotional support needed by their sick child or adolescent and the child's siblings. Parents often launch themselves into intense activity. They rapidly absorb information about the disease and its treatments while protecting the child patient and mobilizing their own support systems.

An important task for parents is deciding when, how, and what to tell their child about the diagnosis. Children, as well as parents, later recall vividly what took place when the diagnosis was revealed. Ideally, the parents should be the ones to share this information with their child. Parents need much guidance in this task. They often use euphemisms and attempt to protect their child (and themselves) from the harsh realities of the diagnosis and the illness itself. They need help in understanding why such information must be presented both honestly and calmly, how (including the timing issue of “when”) to communicate information about the nature of the illness as well as the impending changes in activities, appearance, and energy. Many parents find it difficult to believe that thoughtfully open communication promotes understanding and trust. They need repeated explanation that honest discussion, when properly timed and tailored to the age and developmental level of the child, works best. Such communication avoids the distortions of secrets held, promises not kept, and misinformation given. [14] Family stress can be better managed once the child understands and accepts the diagnosis. This paves the way for more open communication within the family. [15,16] and [17]

**Child's Reaction to Diagnosis and Initiation of Treatment: Developmental Considerations**

A diagnosis of cancer is traumatic for all children, but the child's age and developmental level significantly influence the experience of illness-related events.

***Infants***

The burdens of a cancer diagnosis in an infant fall primarily on the parents and other caregivers. The progression of normal growth and development is dramatically altered. Health professionals need to be very proactive in assisting parents with the many dimensions of bonding to the ill infant. Specific guidance is needed in the many dimensions of infant care, including bathing, nutrition, stimulation, and touch.

***Young Children***

The young child's immediate concerns revolve around hospitalization, separation from parents, and fear of medical procedures. Toddlers and preschoolers are particularly sensitive to separations and changes in familiar routines. These young patients may view hospitalization and disruption of usual daily life as punishment. This perception is further reinforced by the experience of painful and invasive medical procedures.

Hospitalized young children need constant reassurance from their parents that they will not be abandoned and that hospitalization and medical interventions are not a form of punishment. The young child's concerns about body boundaries along with fears of mutilation require special attention in relation to all medical procedures, from temperature taking to lumbar punctures. Before any medical procedure, children should receive a brief, honest description about that procedure. They should be told what the procedure is for, how it will be done, and the intensity and duration of any associated pain. [18] This description should be combined with behavioral interventions that reduce the child's anxiety and distress surrounding medical procedures. [18,19] and [20]

Behavioral interventions include a system of positive incentives in combination with strategies of attentional distraction designed to help the child control fear before and during tests or treatments. The presentation of a valued reward may elicit the extra motivation to cooperate (e.g., to try to remain still). Expectations for the child's behavior must be realistic and must allow the child to succeed.

Children often want to be brave and not fight medical procedures. They welcome the physician's help in controlling their fears, especially when it is presented in concert with the child's primary care nurse and parent. Such help may take the form of attentional distraction, emotive imagery, or hypnosis. During painful procedures, children can be engaged and distracted through storytelling, fantasy play, drawing, cognitive puzzles, and video games. Parents should be helped to develop these skills for use with their children. A more structured desensitization or emotive imagery procedure is most useful when the child's fears are not directly linked to aversive procedures. This technique is first introduced away from the treatment area. The aim is to teach the child mastery of the feared situation through repeated fantasy—for example, imagining how a favorite storybook hero would cope. The fantasy is designed to elicit motivation to master the pain rather than to avoid it. Finally, clinical hypnosis can be used to train the child to refocus attention on images or thoughts that are unrelated to the source of distress. Hypnosis is not appropriate for all children and adolescents. Appropriate evaluation of the child is needed. Those successfully trained in this technique of focusing attention while “letting go” of hypervigilance can gain a sense of improved comfort and control. [21,22] All these techniques succeed by diverting the child's attention away from the feared procedure and toward more positive activity.

When surgery is planned, the child needs special preparation. Visits to the operating and recovery rooms and familiarization with the surgical personnel and surgical garb (i.e., masks and gowns) help to ease the child's fear and to lessen the shock of the strange environment. The child also needs to be prepared for the consequences of surgery. Discussions about amputation or other resulting disabilities or deformities must begin early. This kind of trauma is best handled when children have several days to assimilate the information, to voice their fears, and to adjust expectations in the light of further explanations. Other amputees can help to contain some of the child's anxiety. Finally, as with all intensive and painful procedures, physical comforting (appropriate touch, holding, and massage) is essential.

The stress of illness and hospitalization can cause psychologic regression in all patients. For the young child, regression may take the form of loss of newly acquired

skills (e.g., toilet training, speech, self-feeding). Previously discarded behaviors, such as thumb sucking or clinging, may reemerge. Disturbing dreams or night fears may occur. Parents should be helped to understand this regression as part of the child's efforts to cope. The best approach is to avoid either scolding or ignoring these behaviors. Adaptive regressive behaviors generally subside after the acute phase of illness. At times, regressive patterns may become more entrenched and can become serious problems. For example, although increased dependency between parent and child is inevitable, this can lead to more extreme symbiotic regression, a maladaptive behavior pattern that is often difficult to change given the anxieties as well as misperceptions of both parent and child.

***School-Age Children***

In the school-age child, diagnosis and initiation of therapy arouse many of the same feelings and fears seen in the preschooler. Separation, the presence of strange people, an unfamiliar environment, fears of abandonment and punishment, and threats to body integrity are all major concerns of the school-age child.

School-age children cope with the stress of illness in various ways. They may have a delayed initial reaction or may respond immediately with acute anxiety or panic. Other reactions include psychosomatic complaints, nightmares, labile emotions, regression, and stoic, adult-like acceptance.

School-age children are likely to be verbal about their illness and to request information about all aspects of the disease and treatment. They often pose difficult questions about the reasons for and causes of their illness. Children at this age also experience pride of mastery from the learning associated with their illness. They enjoy learning the proper labels for their disease, treatments, and medications. They can use this information effectively when they return to school.

This is a developmental period of vigorous inquiry. The diagnosis of a severe illness, with all the associated anxiety, prompts a barrage of questions. From the outset, these questions should be answered in a simple, straightforward approach. A sample exchange might be

Q: Why are we going to the hospital?
A: Because Dr. Jones thinks you may have a serious illness.
Q: What kind of serious illness?
A: It may be a blood disease called leukemia.
Q: Does that mean I am going to die?
A: We are going to the children's hospital because they have special treatments for leukemia that have cured many children.

Parents often need to engage in specific scripting because they are not used to conveying stressful information to their children. Helping this process should begin at the intake meeting. If the family's usual practice is to avoid issues, the child will learn that asking questions produces discomfort in the parents. Parental discomfort increases the child's personal distress. Behavioral interventions to enhance management of stress should focus on developing self-regulatory skills, acquired through biofeedback, hypnosis, or imagery training. Here again, parents often are willing participants. Their participation augments both successful learning by the child patient and parental stress management skills.

Once the physician has formed a working relationship with the child, a special education session between physician and child, without the parent, provides an opportunity to correct any misconceptions or to offer more detailed information to the child. Obviously, the amount of detail presented depends on the age, comfort level, and sophistication of the child. This approach may lead to a warm bond between physician and child that can enhance compliance (adherence) with procedures and treatment. Although some parents may feel threatened by this exclusion, most are grateful that the physician is sharing the educational task with them.

The major activity of children outside of the home is school. School begins the processes of working toward independence from parents, establishing peer relationships, and acquiring academic skills. The physical and emotional concomitants of life-threatening illness disrupt school attendance and performance.

***Adolescents***

For the adolescent, caught up in the complex transition between childhood and adulthood, illness presents unique issues. Focal concerns about independence, appearance, acceptance, sexuality, and future plans are immediately confronted.[23] Adolescent strivings toward autonomy and self-determination are inevitably threatened by the forced dependence, compliance (adherence to treatment regimens), and loss of control accompanying the illness and treatment.

Most adolescents are self-conscious about their appearance and emerging sexuality. These issues assume an even greater significance in the face of disease and treatment-related delays in puberty,[24] as well as such physical changes as hair loss, weight alterations, and mutilating surgery.[25] Concerns about infertility may be misconstrued or not addressed at all,[26] may result in fears of impotence or frigidity, and may increase the chances that the adolescent will develop a distorted image of his or her own sexuality. The sense of physical weakness and vulnerability similarly interferes with the adolescent's maintenance of peer interactions. For example, investigators have suggested that teens with cancer are more avoidant and guarded in their male–female relationships than are their healthy peers.[26]

School life is also disrupted. Both social and academic pursuits are interrupted, delayed, or critically altered by frequent and prolonged school absences. Feelings of isolation or embarrassing physical changes may make it difficult for the adolescent to return to school or to maintain adequate attendance.[27] Finally, long-established plans or expectations about career or family may require reconsideration in light of physical limitations, academic difficulties, and questions related to fertility and parenthood.

Each adolescent responds to these stresses differently, and the same teenager may react differently at different times. Initially, some adolescents respond with questions that are general rather than personal.[28,29] Concerns may center on causation and prevention of the disease, new research and treatments, statistics about recurrence and survival, and payment of medical bills. For others, more personal issues, such as the specific treatment plan, the effect of treatment on appearance, and disruption of family, peer, and school activities, emerge as immediate concerns.

Although these specific issues need to be individually evaluated and addressed periodically throughout treatment and follow-up, several initial strategies help to facilitate the adolescent's mastery.[30] The adolescent patient must be allowed and encouraged to participate in medical decisions (including cosigning informed consents). Within the limits of the particular treatment center, the adolescent can be given control over the scheduling of treatments and procedures, permitted to see radiographs or test results, and involved actively in discussions of alternate treatments. Particularly for the older adolescent, these experiences are helpful.[29] Both group support and individual counseling should be available to the adolescent. Finally, parent education should focus on helping parents encourage their children to maintain active participation in daily activities, including school and extracurricular programs. Parents must learn to give themselves and their adolescent children permission to argue, to voice opinions, and engage in normal, developmentally appropriate intrafamily disagreements.

**Family's Reaction to Initiation of Treatment**

A new adaptive equilibrium in the family occurs as treatment is initiated. This equilibrium incorporates the illness, as the family reaches for a new sense of normality. Treatment fosters parental confidence, with a sense of hope for the future.[31] As one mother stated, “After I found out what needed to be done, I felt like I had something to hold on to . . . a chance for a cure. Almost immediately, I felt as if I had some control back . . . now we had something to fight with.”

Along with the feelings of relief, optimism, and improved mood, the initiation of treatment stimulates anxiety, particularly concerning side effects. Eliciting from families their understanding and expectations of the illness and diagnostic and treatment procedures and correcting these impressions as needed, combined with further education and explanations, all become essential activities. Misunderstandings commonly occur at this time because of the “selective” hearing of the parents as well as their assumptions about how busy physicians are. Some parents retain in conscious memory information that reinforces their hopefulness, whereas they fail to recall information with negative implications. Perceiving the physician as “too busy” gives the parent a comfortable reason to avoid confirmation of valid fears about the child's condition. Coping with the treatment process is often affected by the length of the prediagnostic period: a rapid diagnosis, without uncertainty, demands much less of the family's resources than a prolonged prediagnostic period of professional uncertainty. Equally important, he physician needs to reclarify for the family the nature of his or her involvement throughout the treatment process: how much care the physician will administer directly, what will be done by others, and the physician's supervisory role.

**Informed Consent**

Informing parents and their child about treatments is similar to informing them about the diagnosis of cancer. The approach, the setting, the need for repetition, and the awareness of each family's unique strengths and limitations all remain important. Parents fear the complications of procedures and treatments. Especially if the child feels and looks well, parents tend to want to postpone interventions that seem “risky” to them but are considered necessary by physicians. This desire, especially when combined with the unsettled feeling caused by not fully understanding the information presented through consent forms, can further threaten physician–family relationships. Careful explanations of the disease and its treatment that are tailored to the understanding of each family significantly improve compliance. It is a positive sign of coping and adaptation when the family asks questions, openly expresses concern, and actively seeks to increase understanding of the child's disease and treatment.[32] Parents should be encouraged to take notes; we suggest offering them pads and pencils. Taking notes during meetings assists parents in recall and in formulating questions. For parents who are not literate or who speak another language, a team member assigned as a patient advocate or a translator should be present (see the section Cultural Differences). Only the physicians involved in the child's treatment should be responsible for informed consent. Nine steps can assist families through this process[32,33]:

1. A full explanation of the treatment and associated procedures must be presented. The language should avoid professional images and jargon. Professional terms that are unavoidable should be explained in lay images and words.
2. The purposes and expected benefits of the treatments need to be listed.
3. Common morbidities from procedures as well as common morbidities and side effects of treatments should be outlined. Overinforming verbally (e.g., presenting extensive lists of all possible side effects) generates confusion and anxiety. It should be avoided.
4. Alternative (complementary) treatments need to be acknowledged and discussed.
5. At this point, the physician should stop and review both questions and psychologic reactions with the parents. This recognition of the parents' feelings and thoughts invariably enhances their positive reaction to the physician. It also helps to quiet anxiety. This is the time to inquire about the known or expected reactions from grandparents, other relatives, and friends; these caring people may pressure the parents with their own disbelief about the diagnosis, their anxieties, and advice. Learning about such pressures helps the physician to understand the parents' questions and emotional responses more clearly.[32]
6. The voluntary nature of treatment must be made clear.
7. Parental awareness of the right to withdraw from treatment should be explained carefully, including the meaning of withdrawal “against medical advice.” Describe, if necessary, those situations in which the physician will vigorously pursue treatment over parental objections, even to the point of obtaining a court order supporting treatment.
8. The foregoing steps are summarized in the patient's medical chart; at the very least, the date, time, and those present when informed consent was obtained are to be noted.
9. Written consent forms are signed when appropriate or required.

Consent forms almost always cause families considerable stress. Studies reveal that most consent forms “obfuscate, intimidate, and alienate.”[3] The readability of these forms is often at a college or higher educational level.[3] In clinical research settings, the realities of informed consent are compounded by requests to participate in randomized treatment trials. Such trials tend to restimulate guilt in parents and provoke feelings of helplessness, anxiety, and anger.[8,15,34] Parents fear the complications of procedures and treatments. Their desire to postpone these interventions—especially in a child who seems well—coupled with being unsettled by not fully understanding information presented through consent forms threatens physician–family relationships.

Nausea, vomiting, and hair loss are frequent side effects of chemotherapy agents. Infections and toxicity may also occur. Highly visible side effects (hair loss, muscle weakness, ataxia) or severe toxicity (neutropenia with infection) generate guilt in parents about having given their consent for treatment. As one parent described it, “I can't stand watching the chemo being administered. I feel as if I'm permitting my son to be poisoned.” Many parents struggle with the changes in their child's appearance: “I know she's the same person and I love her every bit as much as I always have. But she looks so different. Only a few weeks ago she was standing on the stage of her school play singing. Her hair looked so beautiful and her eyes sparkled so. I can't let her see how much her appearance bothers me. Truly, my biggest consolation is how well she's handling all of this.”

Unfortunately, for some patients, amputation may be the best or only treatment for the disease. Although numerous effects on the patient undergoing an amputation have been noted in the literature, many health care professionals tend to overlook the effect that an amputation may have on parents.[32] The loss of a child's limb is experienced as a loss for the parents as well. Parents mourn this loss, and they need time to accommodate to the resultant disfigurement and to integrate the modified body image of their child. As one mother stated, “I'll do anything to save his life—including an amputation. But I feel as if I'm losing something that is mine too. I love his leg like I love all of him. People don't seem to understand that.”

Parents welcome the opportunity to discuss their feelings about their child. Talking about the changes in their child's condition and appearance as well as the changes taking place in their own lives with someone who knows the child's situation and with whom they feel can be sensitive to their own personal struggles is most helpful.[35] Thus, *true informed consent is a process that extends beyond a few formal meetings and printed consent forms*.

In summary, complexities of the diagnostic and initial treatment period call on all the family's resources and all the clinical skills of the medical staff. Families need assistance in preparing for the long siege of illness. Families need to learn the value of dealing openly with serious illness and the importance of maintaining a balance between the needs of the sick child and those of other family members.[14] Parents who are under emotional stress and who are facing financial burden are apt to neglect or postpone necessary care for their own or the siblings' health. Efforts need to be made to obtain information regarding current health problems of *all* family members. This information can then be used to prevent future crises and to help conserve the family's energies and emotional resources.[36]

Despite severe stress, most family members manifest considerable resilience throughout the disease course. They learn to accept the child's diagnosis and treatments realistically. Many are able to hold a neutral, if not optimistic, view of their lives while acknowledging the life-threatening nature of the illness.[16] Various factors influence how a particular family copes. These include the personality of individual family members, the family's background, how previous crises have been managed, and the current economic and social situation.[11,15,17,37] Coping does not mean absence of problems or severe emotional upsets, or the occasional use of defensive measures and behaviors. Acute stress reactions are normal. Families benefit from being informed that their own responses are understandable and appropriate.

Throughout the disease course, each family demonstrates a range of responses along a spectrum of adaptability. Ideally, problems in adaptation are immediately identified at each stage of the process (diagnosis, informed consent, initiation of treatment). Table 46-3 presents the spectrum of warning signs and problems in adaptation. All maladaptive responses must be identified early and psychosocial intervention obtained. Such interventions may include crisis intervention, insight-oriented psychotherapy, the use of psychotropic medication or other treatments such as behavioral techniques (which address specific problems), and supportive therapy for the family.

The family's initial coping style can be used as a significant indicator of its long-term adaptability.[38] Therefore, it is best to have a thorough understanding of how the family functions before the diagnosis as well as at the time of crisis. Such an understanding allows the health professional to assess the impact of the diagnosis on the family, to identify behaviors that may indicate future problems, and to plan how to offer help most effectively.[31] One approach that is often helpful is to engage the family in predicting how they will cope with future crises. Families then feel forewarned and prepared and are able to adapt more effectively to stresses that occur throughout the treatment process. Evaluation of family functioning is best formulated at the time of diagnosis or on entry into a new medical environment. Such “intakes” need to be ongoing, however, to reassess family functioning at the different stress points throughout the child's disease course.

**Parental Adjustment during the Initial Treatment Period**

A study by Cook[39] found that parents tend to remain at the hospital during the initial diagnosis and first therapeutic interventions. Once the first crisis passes, the mother is likely to assume the burden of day-to-day care of the child. Mothers are likely to stay overnight during subsequent hospitalizations, whereas fathers are often responsible for the care of ill child's siblings and other home-related matters. Mothers continue to remain responsible for outpatient visits.

Decisions regarding a shift of roles in the financial support, management, and care of the home and family appear to be influenced by several factors, including the location of treatment (out-patient or in-patient; close or far from home), the burdens treatment causes, whether both parents are employed, the age of the affected child and siblings, and, most important, the ability of the parents to communicate openly and to share tasks.[40] Open communication allows spouses to negotiate the reallocation of roles more effectively, resulting in a more cohesive, less conflictual family environment.[34]

A mutually supportive marital relationship is a significant variable in the family's ability to cope with the stress imposed by childhood cancer. [41,42] Nevertheless, parents are often reluctant to take time to meet their own individual needs or those of their spouse. [43] Many parents tend to be overprotective and to include the sick child in most of their activities.

Fife[44] identified several reasons for this additional strain on the marriage: (a) the parents' need to “make the child as happy as possible”; (b) a sense of guilt regarding the child's disease; (c) fear of leaving the child alone or in someone else's care (compounded by the fear of permanent separation and loss of one's child); and (d) involvement in their day-to-day stress to a degree that leads them to disregard the importance of their own needs or those of other family members.

The separation of family members from one another (the mother and patient at the hospital and the siblings and father at home) can cause significant stress and may also strain marital ties. [36] Some parents may be apart from their spouses for the first time and thus faced with the difficult task of making decisions independently. When both partners are forced by their child's illness to give up roles that were exclusively theirs before the illness and to assume new ones, problems arise if the parents remain emotionally invested in their relinquished roles. [45] “Dyssynchrony” of coping styles may occur. [46] This often leads to isolation of family members from one another and feelings of abandonment and lack of empathy. [47] For example, mothers often perceive their husbands as being disengaged or disinterested. When differences of coping styles are identified and addressed, tension within the marriage and family is often considerably alleviated. It is also important to become aware of how both parents understand the diagnosis. Do they understand it and its implications in the same way? Was the plan for treatment agreed to by both? Do they have compatible ways of dealing with and adapting to stress? Are they able to understand and support each another? Does one parent believe that he or she is doing all the work while the other feels left out? [17]

The staff must treat each parent as a unique person with his or her own experiences, needs, and roles within the family structure. Attempts at understanding the personal and family history of each parent are essential to successful interventions aimed at preserving family ties. Such efforts at understanding enhance the family's ability and willingness to place trust in the hospital staff.

Because the patient's mother is most often at the treatment facility, her strengths and vulnerabilities can be identified more readily than those of the child's father. Unfortunately, fathers tend to receive less support, have limited opportunity to share their concerns with others, and often feel guilty and excluded from the daily aspects of the child's life and care. When given the opportunity to do so, fathers often describe the difficulty of having to perform at work and at home, of constantly having to alter work schedules for family obligations, of missing life “as it was,” and of feeling helpless. Their most commonly reported concern is for their child's future.[48]

Both parents must be involved in the child's program of care. Overall, fathers are the most vulnerable. Make special efforts to (a) include the father in as many of the early discussions as possible; (b) enable him to express his particular concerns; and (c) help him to become more familiar with the day-to-day responsibilities that his wife will have while looking after their child. [15] At the sametime, attempts must continually be made to keep communication open between the parents. Both parents should feel that they have firsthand knowledge of their child's medical progress and that their involvement is essential to the well-being of their child.

Parents often benefit from support groups in which families can learn from one another how to meet their own needs as well as those of their sick child. They also benefit from individual or family therapy in which the issues of communication, intimacy, or differences in coping styles can be addressed and, one hopes, resolved.[31,47] The Candlelighters Childhood Cancer Foundation as well as the American Cancer Society and its local chapters can be sources of support for families interested in self-help groups within their home community and for those who wish to obtain a bibliography of reading materials and films pertaining to their child's disease. Many other resources exist today. A significant number are readily available through the Internet ( Table 46-4).

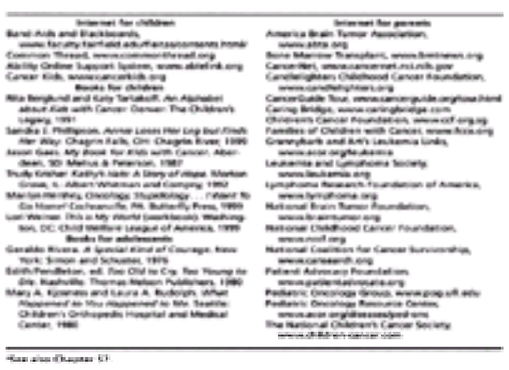

**TABLE 46-4. SPECIAL INTERNET AND BOOK RESOURCES**[a]

## ADAPTATION PERIOD

### Early Remission and Ongoing Treatment

After the induction of therapy, the child often has a period of remission or tumor regression. The child is able to go home for extended periods, returning to the hospital on a scheduled basis to receive chemotherapy or radiation treatments. This process may continue for years.

### Compliance

Ongoing compliance—now referred to as *adherence* by the psychological community—with medical regimens is an important part of living with and adapting to cancer. This includes following prescribed drug protocols, enduring multiple medical procedures, and adhering to appointment schedules. Reports suggest that noncompliance is a significant problem in pediatric oncology, with rates of medical noncompliance ranging from 33% to 59%. [49,50,51,52,53 and 54] Noncompliance is serious; it can hinder or negate attempts to provide optimal treatment and can compromise the young patient's chances of survival.

In addition to influencing therapeutic outcome, noncompliance affects other aspects of cancer therapy; medications may be misjudged as ineffective, unnecessary diagnostic tests may be ordered, and alternative treatments may be initiated. Undetected noncompliance also precludes reliable assessment of new or experimental treatment regimens, resulting in erroneous conclusions. Finally, the extent to which noncompliance contributes to poorer outcomes in certain groups of patients (e.g., adolescents with leukemia) remains an unanswered question.

To date, few comprehensive studies of compliance issues in pediatric oncology populations have been conducted. Several circumstances surrounding the treatment of childhood cancer probably contribute to poor compliance, however. These include anxiety, the aversiveness of procedures, the complexity and prolonged nature of drug protocols, and the patient's age. [54,55,56,57,58,59 and 60]

The aversiveness of medical procedures and treatment side effects, even if accepted initially, may interfere with long-term compliance as symptoms improve and disease remission is obtained. [61] Children with cancer do not habituate to painful medical procedures, and anxiety may only intensify with repeated clinic visits or hospitalizations. [59,60,62,63] Treatment failures may also result in noncompliance as discouragement and hopelessness set in.

Some studies target the adolescent as being at greatest risk for noncompliance. Compared with younger children, adolescents were found to be less compliant with oral medication[53] and, in general, less cooperative with their medical care. [59,60,64] Although the adolescent is beginning to assume some adult responsibilities, these are probably carried out inconsistently. Confusion about responsibility for certain functions increases the chances of missing appointments or medication doses. Noncompliance may also be one attempt to maintain and exert control in the face of the forced dependence and restrictiveness of illness. Alternatively,

noncompliance may represent the adolescent's denial of illness and its life-threatening consequences. [65]

The following suggestions are offered as strategies for enhancing the adolescent's cooperation and compliance with treatment procedures. First, as discussed previously, the adolescent must be included as an active participant in treatment-related decision making. To the extent of the youth's capability, the adolescent should be given as much choice as is possible (e.g., scheduling treatments when they will least interfere with other activities). Increasing the adolescent's sense of control increases compliance. Second, the oncology team can help the family to set clear expectations and to clarify roles. Decisions about who will be responsible for administering medications or for remembering appointments should be made early, before problems arise. In this regard, it can be helpful to both physicians and adolescents to set up a contract, a system of expected behavior and consequent reward. [50,56,57] Third, medical personnel should provide written directions and should make sure that patients understand medication schedules. Another helpful approach is to set up visual or auditory signals to remind adolescents when it is time to take their medication. For example, the patient can learn to identify a daily routine that can be linked to the taking of medication. Finally, if necessary, other family members or friends can be recruited to assume supportive or supervisory roles. A supportive parenting style is consistent with improved compliance. [48]

## Social Reintegration of the Child

Reentry after treatment initiation involves resuming social activities and roles. Specific concerns depend on the child's age and developmental level. Children play several roles in society, each important to the progression of their social development. They are son or daughter, friend, and student; by adolescence they may also be recognized as athlete and boyfriend or girlfriend. These roles and corresponding arenas of social interaction provide the vehicles for the development of mature and independent relationships. Cancer disrupts these avenues of social activity and forces the young patient to relinquish the usual roles, at least temporarily. The new, singular role is that of patient in an unfamiliar system of doctors and nurses instead of participant in family life, peer relationships, and school. [66] The family provides the first social setting in which the child with cancer copes with his or her reaction to serious illness. Thus, the family has the opportunity to set the tone for the child's adjustment. [67] By structuring daily living for the child with cancer in accordance with normal expectations for a well child at a parallel developmental stage, a family can help to alleviate the child's feelings of being alone and different. [68]

The first priority is to reestablish patterns and routines of daily life that were disrupted by initial treatment or hospitalization. For young children, familiar routines revolving around bedtime, toileting, feeding, naps, and playing provide a sense of control and security. For older children and adolescents, attention should be paid to reestablishing the child's role in the family.

Discipline is another area that may be disrupted. Parents often become lax in their expectations of ill children. At the same time, they may become overprotective or overindulgent. If this cycle continues, significant behavioral problems will result. Even ill children need firm and consistent limits, with predictable disciplinary measures if they do not comply with the set limits. Lack of limits being set leads to a sense of lack of control and insecurity in all children, particularly those threatened by a serious illness.

The development of friendships and separation from family are key tasks for school-age and adolescent children. Illness and treatment impose a dependence and loss of control on patients. Dependence and loss of control impede the drive to achieve separation from parents in the school-age child and independence in the adolescent. [69,70,71] and [72] The school-age child typically gains the most significant social contact at school, learning to relate to other children of the same gender and spending considerable time with a constant friend. With the adolescent, close relationships with peers of both genders are important. The youth builds these relationships while struggling with self-definition in social, moral, and physical domains. [74,75] and [76] If treatment causes striking changes in appearance, adolescents may be devastated because body image is so central to their self-esteem. [77,78,79] and [80] Although these disruptions do not necessarily lead to psychopathology, they have potential long-term, significant implications for the progressive development of the child's social confidence and competence. [71,81,82] Thus, maintaining contact with friends is important throughout treatment, even during times of low energy. For example, planning short visits and making telephone calls are compromises to consider when the child is still weak. [66] Today, more and more children use e-mail and Internet messaging services to stay in touch with one another.

A critical research and clinical need exist for the development of appropriate tools to facilitate the monitoring of social functioning in a child who has endured the pain and isolation incurred by cancer and its treatment. Attributes of an effective measure based on clinical experience in pediatric oncology settings include simplicity, brevity, and conventional psychometric properties. [83] Similarly, no consistent, comprehensive, and generalizable protocol is available for promoting the social adaptation of the child with cancer. At present, institutions differ in the number and type of social activities (e.g., playroom, volunteer programs, special camps) and interventions (e.g., child life or support groups) available to the children they treat. The critical next step is to measure the social performance of these children systematically, to follow their progress over time, and to develop interventions to minimize potential developmental interruptions. As such, social skills training represents an emerging strategy for helping these children adapt by gaining support from peers. [84,85]

### *Return to School*

A child's life is organized around school, and successful reentry also demands a rapid return to this environment. School provides for the development of academic abilities as well as peer contacts and social activities. A child who misses as little as 4 weeks of school in a year may encounter problems in building the skills necessary for academic progress. Similarly, a month away from one's peers interferes with the shared experiences that make up friendships.

Missing school has been related to serious stress and adjustment problems. [60,80,86] The illness and its treatment may also be associated with reduced concentration, memory deficits, and speech impairments, [79,80] which further interfere with academic and social learning.

For the child with cancer, school plays a vital role as the most immediate and important part in normalizing the child's life and counteracting the anxiety, depression, and isolation that may accompany illness and treatment. [87,88,89] and [90] Thus, successful rehabilitation must start with the reestablishment of these usual routines.

Children with cancer often have difficulty returning to school and maintaining attendance. [70,91,92,93] and [94] They show high rates of absenteeism and school refusal, missing an average of 21 to 45 days per year. [93,95] Long-term survivors of cancer have reported school absenteeism to be one of the most significant and disruptive consequences of having cancer.

Reasons for the child's missing school extend beyond the unavoidable absences associated with clinic visits, hospitalizations, and treatment side effects. Children must also cope with fears of death, the reactions of others, fatigue, and activity restriction, as well as changes in physical appearance caused by weight gain or loss, alopecia, or amputation. Resulting anxieties and embarrassment significantly contribute to the child's reluctance to attend school. [89] The development of a dependent–protective relationship between the parent and child may also reinforce school absences. [96]

Interventions facilitating the child's school experiences are imperative. [97] Prevention is one of first steps, accomplished by establishing a system for early and ongoing communication among the family, school, and medical personnel. Early detection and treatment of problems are the goals. To be most effective, such a program can begin at diagnosis, with documentation of prediagnostic levels of achievement and attendance and baseline assessment of current functioning (achievement and neuropsychologic testing). Continuity of classwork should be arranged directly through the child's school or, when necessary, through hospital schools or homebound teachers. At the same time, to minimize feelings of isolation, the patient and family can be encouraged to maintain contact with the child's school friends and teachers. The patient, classmates, and school personnel all need to be prepared for the child's return to the classroom.

For the patient, concerns about appearance and the reactions, questions, or misunderstandings of classmates are foremost. Opportunities to rehearse explanations or possible answers often decrease anxiety. It is usually best to tell the child to respond to questions or comments briefly, directly, and honestly. The child's specific response depends on his or her comfort and developmental level.

In addition, teachers can obtain permission from the family to prepare the class in advance for the child's return by describing events openly and answering questions honestly. Alternatively, some children with cancer are interested in talking to their class as a group. Presentations or projects, such as a show-and-tell for young children or science or health projects for older children, may be used to inform the class about the disease and its treatment. [98,99] and [100]

School personnel need information about childhood cancer and its specific implications for school. The importance of successful school experiences can be emphasized and appropriate behavioral and academic expectations discussed. [101] Teachers require a description of the child's medical status, treatment side effects, prognosis, and daily functioning. Specific information about any physical changes or restrictions, potential absenteeism, and treatment schedules further alleviates the

teacher's own anxieties and uncertainties.[89] Conferences, workshops, and telephone contacts all are useful ways to convey this information. Teachers have responded with reassurance and appreciation for such opportunities to ask questions and to clarify misconceptions. [101,102,103 and 104]

***Play***

Play occupies a central role in the mental and physical growth of all children. Serious illness and its accompanying stress and physical restriction interrupt natural play and socialization.[105,106] Specific developmental tasks, such as the toddler's exploratory behaviors or the adolescent's identification with peers, may be diverted. Parents may be fearful of injury or anxious about their child being with other children. An important task for the oncology team is to encourage the child to resume previous play as much as possible and to participate in available supervised experiences.

In many pediatric oncology centers, the child with cancer has access to established supportive activities. These opportunities range from hospital or clinic playrooms to structured groups to special summer camps. The child's participation in such play and recreational programs is important beginning at diagnosis and continuing through treatment and remission to long-term survival. Over the course of illness, these activities may assume varying functions, helping to prepare the child for medical procedures, forestalling major developmental disruptions, and facilitating the child's reentry into the community of peers.

Hospital and clinic playrooms provide the patient with a child-centered environment. They offer a safe place, a setting free of medical procedures in which the patient can restore, in part, normal aspects of living. Play activities provide a needed source of pleasure and a medium for self-exploration and expression. They offer the patient an opportunity for mastery and control as opposed to the passivity and dependence enforced by illness. Play may reduce anxiety by helping the child to overcome fears and to cope with frustrations. Finally, playroom activities encourage the social interaction that is particularly important in counteracting the isolation facing the child with cancer.[107,108]

**Parental and Family Adaptation**

Given the improved survival rate in childhood cancer, health care professionals emphasize normality throughout the treatment and adaptation period. This approach creates a “burden of normalcy”[8] as the family is faced with the task of reorganizing itself, changing previous priorities and expectations, and reassigning roles. Optimally, both parents are physically and emotionally available to share the responsibility of the child's care. Unfortunately, this is often not the case. The increased burden of care falls more heavily on one parent.[40] Families who live in communities without a major cancer treatment center face additional problems: the need to travel long distances for treatment; separation from home and most supports during a stressful and frightening time; and the strain on finances resulting from transportation, child care, and accommodations, all combined with rising medical costs.

Particularly when the initial hospital admission has been lengthy, families anxiously await the day their child is well enough to return home. Some parents, however, find this time particularly stressful. The hospital is often perceived as a safe environment, a place where the child's medical needs are continuously met. For the first time, parents may feel powerless and may question their ability to care for their child's physical well-being.

Once the child is home, parents attempt to sustain as normal a life as possible within the confines of the diagnosis. [109] A new day-to-day routine must be established, encompassing the needs of the marital relationship and the well siblings in addition to those of the sick child. When the affected child is confined at home because of fatigue, pain, low blood cell counts, or low morale, stress within the family increases considerably. Parents may not be able to return to their work or to meet other commitments. At the same time, the child, probably bald and possibly cushingoid or disfigured in other ways, must confront the reactions of siblings, relatives, neighbors, and peers. These challenges are most dramatic for adolescent patients.[110] Parents, concerned about the responses of others, may not receive the comfort expected from relatives and friends. This problem may be attributed to fears surrounding separation and death, anxiety about not knowing what to say, or misconceptions about cancer in general. As a result, parents may feel that they can no longer relate to some of the people they had previously relied on for emotional support. A sense of angry disappointment and feelings of isolation may evolve into withdrawal, further threatening the family's equilibrium. This situation can be avoided by specific interventions. Parents benefit from having staff members and other families prepare them for the possible reactions, ranging from support to avoidance, that they may encounter from others. They also profit from having the opportunity to “rehearse” how they will describe the illness, treatments, and prognosis to family, friends, and school personnel before their child's hospital discharge.

When it is time for the child to return for further treatment, some parents resent having to yield some of their parental responsibilities to the medical system again. Others look forward to being assured that their child is doing well or will be receiving treatments that can cure the disease. These responses are appropriate. Soon, most families become actively involved in the treatment process. This involvement entails settling into the routine of the hospital or clinic visits while obtaining a comprehensive grasp of the medical treatments and procedures. All people have their own way of handling stress and coping with crises. Some parents may be perceived as a “tower of strength” throughout the diagnosis and early treatment period, only to “fall apart” temporarily after successful induction therapy. [31] This should not be mistaken as maladaptive behavior. Rather, it occurs because some people, once the initial impact of the disease has been absorbed, find themselves only then becoming aware of the chronic, life-threatening nature of the disease. At this point, parents may begin to mourn the loss of their child's health and their previous lifestyle. Families find the support of relatives, friends, and faith, as well as the honesty of the physicians and nurses, helpful. They often benefit from talking to a nonmedical person about the disease—a person who can help them to anticipate and to cope with the hospital system and treatment. Parents also profit from talking to other parents who have been through a similar experience, from learning to take one day at a time, and from talking with other children who have done well.

The continuum of coping responses seen during the reentry period is described in Table 46-5. Specific factors correlate with family coping mechanisms during the period of remission.[111] These include (a) open communication about the illness within the family; (b) an attitude of living in the present; (c) lack of other concurrent stresses (marital, financial, illness of other family members); (d) the quality of relationships among family members; (e) previous adaptive coping with the illness; (f) coping of other family members; and (g) adequacy of the support system. During remission and treatment, maladaptive coping often occurs in families who had significant difficulties coping at the time of diagnosis. Maladaptive coping may be expressed through many symptoms. These include an obsessive concern about relapse and death; inability to allow the child to return to everyday activities; interpersonal strife; continuous crying and generalized anxiety or worrying; behavioral symptoms in the well siblings; poor compliance with clinic visits or treatments; refusal to interact with other patients or families; and an ongoing pessimism about the unfairness of life in general. In more extreme situations, magical thinking, regressive forms of behavior, or withdrawal from reality may be evident ( Table 46-5). Interventions during remission need to be tailored to the specific needs of the individual family members. Families who have adapted well can benefit from individual or family supportive counseling to find ways in which the family can further unite and develop even stronger ties. In contrast, poor adaptation necessitates ongoing intensive intervention by an experienced mental health professional, to provide the individual family member or the family as a whole with support, limits, and structure (Table 46-6).

| Adaptive responses | Early signs of problems in coping | Maladaptive responses |
|---|---|---|
| Ability of parents and other family members to communicate openly about the illness and how it is affecting their lives<br>Return to work and school, family and community life; making plans for future<br>Ability to assist patient with transitions; social acceptance, self-image, and health-enhancing behaviors while engaging in normal limit setting<br>Ability to call on medical team, other family or community resources for any needed support | Estrangement among family members and inability to establish a comfortable "new normality" within the family<br>Feelings of estrangement; having to force selves to return to work, school, family, and community life; refusal to think about future<br>General pessimism; difficulty in following through on the various parental responsibilities; fear of setting limits; feeling of "walking on eggshells"<br>Little to no spontaneous interactions with support networks, combined with feelings of loneliness and isolation | Interpersonal and intrapersonal disequilibrium evidenced by behavioral problems in well siblings, difficulties in problem solving, and marital discord<br>Inability to return to work, school, family, and community life; estrangement, isolation; no plans for future<br>Withdrawal from parental responsibilities, especially when ill patient, or overindulging patient in every way<br>Avoidance of medical staff, family, and community supports |

**TABLE 46-5. SPECTRUM OF ADAPTATION DURING REMISSION**

| | |
|---|---|
| **Education** | **Psychiatric interventions** |
| Disease and its management | Family therapy |
| Care activities of daily living, finances, nutrition, physical activity, touching and holding | Feelings<br>Communication<br>Dealing with separation<br>Dealing with regression<br>Goal setting<br>Preexisting conflicts |
| Stress management training | Marriage counseling |
| Mobilization of resources and environmental change | Feelings<br>Role changes<br>Case management |
| Transportation or travel | Individual psychotherapy |
| Day care | Anxiety |
| Employment problems | Depression |
| Self-help groups | Working through feelings of helplessness and hopelessness |
| Education resource information | Enhance strengths, promote growth |
| Isolation or legitimization | Group therapy |
| Channeling of feelings and advocacy | Feelings of isolation or support<br>Helplessness<br>Separation<br>Goal setting |
| | Behavior therapy and hypnosis |
| | Anxiety<br>Phobias<br>Sleep disorders |
| | Medication |
| | Anxiety<br>Panic reactions<br>Depression |

**TABLE 46-6. ISSUES ADDRESSED IN INTERVENTIONS FOR PARENTS AND SIBLINGS DURING DIAGNOSTIC AND ADAPTATION PERIOD**

In time, most families develop a new kind of stability in their lives. They become more hopeful that the remission will last and may again make plans for the future. An infection or unexpected treatment side effect necessitating hospitalization interferes with family adjustment. [112] Family members briefly find themselves experiencing once again many of the feelings they had at the time of diagnosis. When the physical health of the child is stabilized and time between hospital visits is lengthened, families may then begin to worry that the details of their child's “case” have been forgotten. Such feelings may be more exaggerated in teaching hospitals in which physicians frequently rotate assignments. These feelings are surmountable. Most families are eager to have the support and understanding of their physician and like to be thought of as special. [113] Therefore, attention must also be given to the emotional needs of families whose child is in remission. Systematic referrals to community-based advocacy and support services (e.g., Candlelighters Childhood Cancer Foundation, American Cancer Society) and use of Internet resources ( Table 46-4) aid in adjustment during this less stressful but difficult phase.

### Parental Expectations and Discipline

As treatment continues, fears related to the disease become less prominent, and other concurrent stresses are perceived as more troublesome. [114] Mothers and fathers often find themselves in a quandary as to how to “parent” their own child. Parenting the child or adolescent in a “normal” way requires parents to control their fears enough for them to return to modified pre-illness expectations of achievement, independence, and responsibility. Doing so allows the child to become a well-functioning and responsible adult. [115] Feelings of uneasiness, guilt, and anxiety about the disease and possible relapse may interfere with the parents' ability to act in the child's best interest.

> “I thought because his treatments were going well, he should be able to continue football practice and be as content and easygoing as before. It wasn't until I found him alone and sobbing one day that I realized that my expectations were unrealistic. . . . I just wanted him to be normal . . . like before.”

> “I remember when I turned 15 and my mother took me to the Social Security office. We didn't need the money but she told me that it was better to apply for disability now so I could be assured of an income later on. I assumed this meant that I could never be well enough to work and that I would be permanently sick. She was wrong—there are plenty of things I can do. . . . That wasn't fair.”

Many parents find themselves feeling frustrated when faced over long periods with excessive dependency by their children. [116] At the sametime, the naturally worried parents, through their vigilance, tend to encourage dependency, to overindulge or overprotect their child, and they find it difficult to administer any discipline. This situation exacerbates the child's perception of himself or herself as different and “singled-out” and places the child in an uncomfortable position with peers and siblings, who resent the extra attention. This extra attention also may be perceived by the child as meaning that the prognosis is worse than he or she had been told. Children may encounter overprotectiveness in one parent and overindulgence by another, or both reactions alternately in the same parent. These inconsistencies challenge the child's understanding and coping skills. [117] Children and adolescents with a chronic or life-threatening disease, reacting to the long-term vigilance of their parents, become especially sensitive to changes in their parents' behavior and attitudes. These changes define the “atmosphere” within which children understand and cope with their disease. The child has already lost his or her health and is fearful of losing parental love as well. When a parent becomes uncharacteristically lenient or upset, one may hear the child playfully request that the “real” parent return. Children with cancer and their healthy siblings often feel relieved when the discipline and structure of the pre-illness period resumes, because it brings with it a sense of normality and parental control missing from their current life situation.

Parents appreciate the suggestions of physicians, social workers, nurses, and other parents concerning their child's behavior. They need to be informed of what the child can and cannot realistically do in comparison with abilities before the cancer diagnosis. [118] Included here are decisions about returning to school and participating in outside activities. Parents need to be encouraged to allow the child to live as full and as normal a life as possible within the restrictions imposed by the disease. They need to find a balance between overindulging the child and setting too many limits. [119] Parents feel less guilty saying “no” to their child when limit-setting has been sanctioned by the physician or other significant persons on the health care team. Parents can reinforce positive adaptation skills in one another through participation in support groups.

The point of completion of therapy and discharge to long-term follow-up is a significant milestone. After successful completion of therapy, the family may be full of joy, pride, and a sense of accomplishment. They may also experience a concomitant sense of anxiety, sadness, and fear, however, recalling other patients who have had relapses or have died. Separation from the treatment team, on whom they have depended for so long, generates uneasiness. After the initial relief of no longer being in treatment, uneasiness and fears begin to grow in parents and the older adolescent patient because they are no longer doing anything to fight the cancer.

Families require additional education and support at this time. Parents and the older child who has completed treatment for cancer benefit from a review of the treatment protocol and the reasons that therapy is being discontinued. They need to be told about treatment options that will be available if the disease recurs. At this point, the physician and other staff members can be especially helpful by explaining to the family the meaning of the word “cure,” by discussing the follow-up care that is provided, and by reviewing symptoms that should be reported without delay. Changes and growth that have taken place in each family member should also be discussed at this time, encouraging an awareness of achievement while preparing the family for the challenges of long-term cancer survival. Finally, patient, siblings, and parents should be instructed about and given written guidelines for self-care.

## RELAPSES AND RECURRENCES: A SECOND CRISIS

Although an increasing number of children with cancer are able to achieve and maintain freedom from disease, some inevitably experience a relapse or recurrence of their disease. In certain ways, this second crisis can be more devastating than the initial stress of diagnosis. [120,121]

Parents often describe the first relapse or recurrence as emotionally the most difficult time, especially when treatment appeared to be going well and no obvious symptoms were present, or when the remission was of long duration. Denial of the illness and fantasies of cure become much more difficult to maintain. After confirmation of a relapse, feelings of shock, anxiety, disbelief, fear, guilt, anger, and sadness are common. Families faced with reinitiation of treatment must start over again, but with a smaller chance for successful outcome. The crisis and stress of the diagnosis are reactivated, the threat to life is relived, and new adjustments are required. [119,122] Encouraging the family once again to adopt a positive attitude toward treatment is a challenge to the oncology team. Families can gain a sense of hope from the knowledge that further action will be taken against the disease. The period of relapse or recurrence is another time when optimal communication among the child, family, and oncology team is essential. Yet feelings of guilt and failure may hamper communication. Support must also be given to staff members who have worked most closely with the child and family. More than ever before, parents require the availability of team members.

Krulik[123] refers to the time between the first relapse (recurrence) and second remission as the midstage of illness. Unfortunately, some children do not survive past this point. Hopes for another treatment response are rekindled when the family is encouraged to begin reinduction or another treatment regimen promptly. [121] Attempts to recreate stability and equilibrium within the family are difficult. Intensive treatments once again limit the family's time for other activities. Work habits, social activities, friendship patterns, relationships within the family, and expression of feelings are again altered. [1] Within the hospital environment itself, the family may experience a change of identity because they are no longer part of the “successful” remission group. [123]

The family may also feel a change in the attitudes of health care team members toward the child and toward them. Team members may be struggling with their own feelings of disappointment, frustration, sadness, and possibly defeat.

When the disease recurs, relatives and friends may encourage families to seek other treatments or new second opinions or to try an unorthodox method of therapy. Newspapers, magazines, the Internet, fund-raising events, and television talk shows disseminate information about cancer research “breakthroughs” and unconventional treatment in such a dramatic way that it is difficult for the general public to evaluate these reports (see Chapter 54). At the same time, the physician may decide that it is in the child's best interest to be referred to another treatment center for participation in a particular randomized clinical trial. The request to consider such a referral can challenge the trust between family and physician, particularly if the family interprets the referral to be a dismissal from care because their child's case is now “hopeless.”[124] This misunderstanding can be eliminated and confidence in the professional relationship reestablished when a pattern of open, honest communication is encouraged and maintained. The physician needs to reassure the child and family that the relationship that he or she has with the family will not be severed.[124]

In most instances, parents continue treatment with their current medical team and refuse to subject their child to unproved methods. Nonetheless, they may experience guilt and anxiety about rejecting a possible “miracle cure.” The treatment team can help to minimize this stress by discussing with the family any treatment information they have received. A commitment to care for the emotional needs of the patient and family, to provide pain control if necessary, and not to abandon the family if the disease progresses, is essential. Each family searches for ways to cope with the renewed threat to life and to emotional equilibrium. At relapse, increased psychosocial support, exploration of the family's strengths, and focusing on enhancing quality of life together help many to rediscover hope and courage. [119]

Most families manage to cope adequately through the different treatment processes. Once again, they need to develop a new sense of normality and stability in their lives. The degree of stability depends on treatment side effects, on the length of time the child needs to remain hospitalized, and on such concurrent stresses as financial pressures, career obligations, and family problems. The altered prognosis elicits feelings of sadness and fears of separation and loss; yet an investment in “going on” persists. Maladaptive coping is manifested by an overly pessimistic attitude about the future that may immobilize parents in their day-to-day functioning. Emotional or physical withdrawal from the child, inability to normalize the child's life, and refusal to follow through with medical care are other signs indicating the need for immediate intervention (Table 46-7). Crisis intervention with individual or family sessions can help the family to alter maladaptive coping behavior.

| Adaptive responses | Early signs of problems in coping | Maladaptive responses |
|---|---|---|
| Accepting as normal the reactions of shock, anxiety, disbelief, fear, guilt, anger, and sadness; ability to express and share these feelings within the family<br>Mobilization of selves and the family system to understand the new medical situation and support the patient in treatments directed at obtaining a new remission | Continuous crying; ruminating about all the fearful possibilities that could occur; sleep disorders; withdrawal from one another<br>Slow response to news of relapse and pessimistic attitudes; absence of information seeking or questioning behaviors | Withdrawal emotionally and physically from sick child or other family members; blaming others for the relapse or other angry scapegoating<br>Withdrawal from all support with refusal to try for new remission despite medical advice; immobilization with or without clinical symptoms of anxiety, depression, severe sleep disorder; inability to deal with daily responsibilities |

**TABLE 46-7. SPECTRUM OF ADAPTATION DURING RELAPSE**

If another remission is achieved, the termination of active treatment often activates a crisis that requires additional education and support from the staff. Parents fear another relapse and the lack of future treatment options. The treatment team must remain in close contact with the family. Not only is frequent medical follow-up required, but the quality of the family's life also needs continual assessment as the family copes with fear and tremendous uncertainty. [117]

Some parents do pursue complementary medicine and unproved methods of treatment or faith healing in addition to, or instead of, traditional or conventional care. For the child with a good prognosis, the health care team should present to the family all the information about achieving that prognosis using conventional treatments. Should parents remain adamant in their refusal to pursue conventional treatments, one should enlist the help of extended family members as well as clergy, if such action fits with the family's religious or spiritual beliefs, as a way of convincing them not to turn their back on conventional care. [125]

Use of the child abuse and neglect statutes is, on infrequent occasions, the only way to ensure that a child with a treatable disease receives appropriate therapy. Such measures should be taken only after all efforts have been exhausted to secure the parents' willing participation in the treatment regimen (see Chapter 53). When the prognosis is poor, regardless of conventional treatment, and the parents seek unconventional treatment or faith healing, review of the illness with the family is again indicated. Even if the parents pursue alternative treatments, they should be reassured that the medical care team remains interested in them and the child's welfare and is willing to provide any care needed.

Important interventions during this time include providing extended family and community supports, creating an atmosphere in the hospital and at home in which family members can talk through their concerns and decisions can be made, and making the entire oncology treatment team available for support, information, guidance, and encouragement.

### Treatment Refusal

When the child (usually the adolescent) refuses treatment, the underlying motivations include hopelessness about the outcome, feelings of helplessness, distress about the side effects of treatment, or a combination thereof. By refusing treatment, some adolescents are asserting their independence and are demonstrating that they are in charge of their own destiny. This is another situation in which preventive measures are far more effective than trying to intervene in a crisis. Preventive measures involve including the adolescent in the discussion and decision-making process from the beginning. Participation in a group for teenagers is also effective because the members confront one another when poor decisions are made, just as they share coping skills with one another and general support. When an adolescent actively refuses treatment, the treatment staff needs to calmly sort out which factors are influencing the decision and proceed in an orderly manner to discuss them. Sometimes the patient feels that the parent is making all the decisions. The patient's refusal is an attempt to assert himself or herself. This situation can usually be handled in a straightforward manner by asking the parents to withdraw a bit to give the adolescent the primary role in communicating with the staff. For patients and parents who believe that treatment is hopeless, the risk to benefit ratio of further treatments must be presented clearly, particularly when the treatment is palliative. Quality of life must be discussed and examined. The distress and discomfort of side effects are the most common reasons for refusing further treatment. They are the most difficult for the adults (the family and the treatment team) to deal with because when challenges are raised to the level of treatment refusal these challenges add to the adults' sense of loss of control. The adolescent, however, is dealing in the here and now, not in the future, struggling to control things in the moment.

A mental health professional not directly involved in the child's care can be of assistance in such instances. [126] In addition, careful evaluation and creative approaches to management of symptoms become important interventions (e.g., the venue of the chemotherapy can be changed). Other ways to enhance the adolescent or child patient's sense of control include teaching relaxation training skills, especially for patients with severe anticipatory nausea and vomiting, and other interventions from the world of complementary medicine (see Chapter 54).

### When Treatments Are No Longer Effective

The course of cancer for some children and adolescents remains a series of treatment responses and relapses leading to a time when established treatments are no longer effective. A few patients remain refractory to all treatments. A time comes for these patients when the continuation of aggressive treatment serves only to increase or prolong suffering.[32] Factors that influence the recognition by parents and professionals of this time include the specific form of cancer; the length of time child and family have dealt with the disease; the child's physiology; the child's and family's threshold of tolerance for physical pain and loss of control; their levels of hope and hunger for life; fatigue; and religious beliefs. [127] Physicians, nurses, psychologists, and social workers generally have no special training in acknowledging and handling this powerfully challenging moment in the history of a patient's illness. The need for such training is underlined by the findings of a study at two different

Harvard-affiliated institutions in Boston (Children's Hospital and the Dana-Farber Cancer Institute). That study [128] found considerable discordance between the parents' reports of their child's symptoms and the documentation of the symptoms by physicians. The physicians and parents were found to communicate poorly with one another about the degree of suffering of the child. This resulted in the children receiving particularly aggressive treatment at the end of life and experiencing substantial suffering in the last month of life.

When the physician believes that the time to offer palliative care has arrived, a meeting with the family should be held. The clinician needs to first elicit from parents their understanding of the situation, selectively sharing impressions with them. The child may or may not attend this meeting, depending on his or her age, developmental stage, and other circumstances. If the child is not present, the physician or parents should later broach this issue directly with the child or adolescent. Over the course of a few meetings, the patient and family need to be told what may happen, physically and emotionally, if they choose to stop all treatments (e.g., when and how the child may die). The staff members who have been most intimately involved with the child and family during the course of the disease ideally should be present at these meetings if the parents wish them to be present. The use of hospice care should be raised. Once the family agrees to end treatment directed at remission or cure, treatment should transition to palliative and hospice care. [127,128]

Once they understand that treatment is no longer effective, parents begin the process of accepting that their child will die. They may experience preparatory (anticipatory) grief. Many of their thoughts focus on preparing for death (this may include rehearsing the funeral in their imaginations) while continuing to hope for cure or recovery.[129] Parents become increasingly vulnerable during this period to the promises of nontraditional or even fraudulent healers and healing rituals as well as to misguided advice from Internet sites and “chat rooms.” The opposing forces arising from experiencing moments of hopefulness while simultaneously thinking of the child's funeral generate guilt. Guilt can be diminished in parents by simply informing them of the normality of these responses. [130]

Hope can be redefined by redirecting energies toward providing as good a quality of life as possible for as long as possible, followed by as good a quality of death (absence of anxiety and pain combined with the presence of loved ones) as possible. Comprehensive care for the dying child involves maximizing physical and emotional comfort. Open communication, pain control, involvement with friends and family, distractions, and the maintenance of familiar routines all convey a sense of security that is important in reassuring the dying child. The family itself needs ongoing emotional support as well as specific information and assistance with difficult decisions and preparations. This is where the many excellent hospice staff can be extremely helpful (see Chapter 51). Painful decisions must be made regarding home versus hospital care for the dying child, autopsy, and funeral arrangements. The decision-making process is facilitated through open discussion ahead of time.

**Talking to the Dying Child**

Struggling with their own anticipations and fears of separation and death, the family needs assistance in refocusing on their child's thoughts and concerns. Many parents are unable to discuss the imminence of death with their child. [131] Parents often believe that the child is unaware of the prognosis and approaching death. Two opposing modes of communication at this time are described in the literature. [132] These are labeled “*the protective approach* in which the ill child is shielded from knowledge of the disease diagnosis and prognosis; and, *the open approach*, which encourages providing an environment in which the child feels free to express concerns and ask questions about his or her condition.” [133]

In the past, it was frequently assumed that children did not understand death and that creating an atmosphere of cheerful normality would protect the child from the seriousness of the illness as well as from the awareness of death as a possibility. Nonetheless, research has shown that children who exhibited a higher level of adaptation to the illness were members of families in which open discussion was allowed and maintained. Waechter [134] conducted a study of hospitalized and fatally ill children and stated that giving a child the opportunity to discuss issues related to death does not heighten anxiety. These findings support the prediction that “understanding, acceptance, and conveyance of permission to discuss any aspect of the illness decreases feelings of isolation and alienation from parents and other meaningful adults and gives the child the sense that his or her illness is not too terrible to discuss.” [134]

In published accounts, parents themselves have documented with great feeling the self-awareness of their dying children. Well-known examples include books by John Gunther, Doris Lund, Mickie Sherman, and Nancy Roach. [135,136,137 and 138]

What do we say to the dying child? How do you help? Children generally have two main questions. The first is, Am I going to die? When the answer is understood to be yes, the second questions is, When? It is helpful to point out to the child what can and cannot be done regarding the illness. Telling the child that cure is no longer a possibility is the most difficult but also the most important message to convey. It is easy for caregivers to camouflage difficult messages in professional jargon. One must give the child this particular message in an open, straightforward manner without going to the extreme of appearing uncaring. The child needs at this time a feeling of security and trust maintained through honest communication.

In telling dying children that a cure is no longer possible, one must also leave room for hope. Hope is redirected from cure to comfort. Comfort includes having people around they love, being free of further diagnostic or treatment procedures, and having pain controlled. Although adolescents may need some time alone, one of the greatest fears of young patients is being abandoned by or separated from family and friends. If these children are in the hospital, a nonrestrictive visiting policy for family should be provided, and interaction with friends and other patients should be encouraged. Even children cared for at home need repeated reassurances that they will not be left alone. Providing comfort also involves acknowledgment and acceptance of the range of feelings that come and go. Children should be told that it is all right to feel confused, sad, or angry—and to talk about these feelings, or, at times, to remain silent. To the extent possible, children should be encouraged to participate in normal daily routines. Continued attendance at school (even if part-time) and involvement in family functions counteract boredom and boost quality of life. Each day can be organized so even children confined to bed feel they are important contributors to their world. Preserving familiar behaviors and schedules also serves to minimize feelings of being a burden.

When given the opportunity, children frequently ask many questions. These often ask (a) what will death be like; (b) what will happen to them after they die; (c) if the “bad things” they have done or thought will cause them to be punished; (d) whether their parents will be all right after their death; (e) when they will again be with those closest to them; and (f) whether they will experience much pain while dying. Parents benefit from being informed of these thoughts. Parents also benefit from exploring their own spirituality. Such explorations, with or without the help of clergy, provide strength to parents as they provide support for both their dying and their healthy children.

Depending on the child's religious upbringing, other spiritual concerns and questions may arise. For example, children experiencing considerable guilt and conflict or feelings of isolation may become frightened and preoccupied about whether “the devil is in their heart” or whether “God will stop watching over them.” Consultation with a chaplain specializing in work with terminally ill children can allay the child's fears and bring the child and family a renewed sense of comfort and peace. Parents often need help understanding their child's questions and providing answers at a level consistent with the child's developmental stage and knowledge of the disease.[139] Some children keep most of their thoughts about death to themselves. This may be due to fear of emotional abandonment by family members and significant others or that their awareness adds an unbearable emotional burden to parents and siblings. [140] Through play, art, drama, and therapeutic conversation, mental help professionals can ascertain the child's private perceptions and concerns and can correct distortions, dispel fantasies, and promote self-esteem through mastery of fears.[141] Parents should be encouraged to participate in such processes.

As death approaches, it is important to help families to believe that they have done all they could for their child. Parents trying to hold on to any semblance of control may seem less cooperative or easily frustrated and annoyed. Such responses are appropriate given the sequence of experiences leading to the terminal phase of illness. Living with dying is a significant additional stress for families. [142] One needs to respect each family's readiness, delicately balancing life issues with those related to palliative care, death, and loss. The medical team's participation and investment in caring for the dying child is extremely important to and greatly appreciated by all families, even those who appear to be coping well on their own.

The terminal phase of illness is an especially crucial time to involve all significant family members. As separation anxiety is heightened, feelings of helplessness and despair may prevail. Family members often find it helpful to participate in the child's physical and emotional care. This care can take place in the hospital or at home. It can include everything from having a sibling help the child eat to having a parent administer medications and oxygen. Family members vividly recollect these terminal events. They can either be plagued by them or find solace in their remembrance. [132] Parents, siblings, and others close to the child benefit greatly from having someone available with whom to share their thoughts, fears, and concerns, whether rational or irrational. Sensitive assistance should be given to the family with difficult decisions and preparations. Parents need repeated reassurance about the importance of their vigil with the child and how this vigil reduces their child's feelings of isolation and abandonment through the moment of death. Home hospice staff usually can provide assistance and guidance to parents and other family members.

### Pain Control

Of utmost concern to all children and parents is how much pain they will have to endure. One of the child's most frequent questions is, Will it hurt? This is asked about medical interventions and is introduced again in discussions about dying. It is the responsibility of the health care team to guarantee the child the most effective pain management available (see Chapter 43 and Chapter 51). This means freedom from intense pain without unnecessary sedation. Because anxiety and pain often occur simultaneously, both factors must be treated. When properly used, appropriate combinations of psychological, pharmacologic, and invasive anesthesia techniques (e.g.. nerve blocks, extradural catheters) can eliminate all suffering from pain.

During preparation for painful procedures, younger children (aged 3 to 6 years) respond to playful interactions that involve their imagination and hypnosis-like suggestions. Older children can be both distracted and involved in verbalized fantasy with a caregiver. [143] Proper pain control enables the child to enjoy the environment actively. At one stage, it may enable the child to attend school; at a late stage, relative freedom from pain makes it possible for the child to interact with others at home or in the hospital.[140] The following is a summary of the principles of effective pain management for the child. [20,144,145,146,147,148,149] and [150]

1. The child may have difficulty describing pain in ways that are immediately meaningful to the physician; determining the source and severity of the pain may thus be complicated.
2. Analgesics should be administered on a regular basis before the effects of the previous dose have stopped.
3. When nonnarcotic agents are no longer effective, narcotic analgesics should be used; addiction in terminally ill patients is extraordinarily rare; physiologic dependence easily managed.
4. Pain may be intensified by sleep disturbance, anxiety, or depression. The judicious use of adjuvant psychotropic medication (e.g., hypnotics, benzodiazepines, methylphenidate tricyclic and selective serotonin reuptake inhibitor antidepressants, or neuroleptics in low doses) improves pain control.
5. When psychoactive drugs are used with children, baseline blood workups (hepatic, renal function) and careful monitoring of side effects (e.g., electrocardiograms if potentially cardiotoxic agents such as the tricyclics are used) are crucial.
6. The child may respond with discomfort or fear to common drug side effects (e.g., the grogginess or disorientation associated with some sedatives); attention to the child's unique experience is important. Elicit that information from the child or adolescent, because it is frequently not volunteered.

## END OF LIFE CHALLENGES: ON PASSIVE AND ACTIVE EUTHANASIA

“Helping a child on his way” and “ending the child's suffering” are sometimes whispered or unspoken issues during the terminal stage of illness. More open and frank appraisal of these issues by health professionals with respect to adults with terminal illness is finally occurring. This more open discussion does not yet apply to children. An influential pediatric psychiatrist at a major university medical center once said, “I consider active euthanasia unthinkable. It implies an unwarranted assumption of infallibility on the part of the physician (spontaneous remissions have occurred in the sickest patients).” [32] Yet, in referring to heroic measure to save a comatose terminally ill child, he also stated, “Passive euthanasia (negative euthanasia) is a different matter.” [32]

Physicians of terminally ill adult cancer patients may offer them the option of shortening their suffering. This may be accomplished through instructions not to treat new infections, not to use resuscitation, to withdraw steroids in patients with CNS tumors, to decrease or remove supplementary oxygen, or to use very-high-dose narcotics to remove the patient from suffering from pain. Similar approaches are known to be used at times by those treating pediatric oncology patients. These realities are rarely openly discussed.

Although many health care professionals agree that children should not be allowed to die in agony, children do die that way. [128,147] A physician and the health care team ideally should be open to parents who wish to inquire about helping their child die in comfort. The physician can serve as a special listener, selectively interpreting and responding to the parent's concerns about suffering. When a parent wishes to ease the pain of dying through more aggressive use of pain medications or through the removal of those treatments that slow the pace of the dying, the physician must examine parental wishes in the context of physician's knowledge of the child's clinical status. If the physician finds his or her perceptions concordant with those of the parent, we believe it appropriate to stand by that parent, to be an advocate for the needs of the dying child while adhering to local laws and medical ethics. Parents obviously have more control in these situations when their terminally ill child is at home or in a residential hospice. After the child's death, the involved professional needs to be available to the parents. It takes time for them to absorb the reality of a passive euthanasia decision into their lives and values. Coming fully to terms with their decision may, like the mourning process, extend over several years. Actions of passive euthanasia are taken out of love and perhaps a feeling in some that they do not have the “infinite strength” to stand vigil over the terminal suffering of their child. Be aware that the echoes and emotional doubts over these actions linger for extended periods of time. As health professionals, we should be available to listen to the parents quietly and acceptingly.

### Bereavement in the Family

The death of a child is one of life's great tragedies. It disrupts a family system in multiple ways. In our society, the bereavement process may have even greater consequences than in earlier times because of the absence of general familiarity with death and its rituals. Death in children accounts for less than 5% of mortality in the United States.[151] Cancer deaths account for 18% of that pediatric mortality. [151] This means that families who lose a child to cancer are an isolated minority, with relatively few social supports for their grief.

The child's death marks the major milestone in a bereavement process initiated when the cancer diagnosis was first heard. Varying degrees of family disruption consequent to such a death have been identified. [142,152,153,154] and [155] Included are rates of marital separation and divorce ranging from 23% to 60%. [156,157] One study comparing bereavement in parents with bereavement over the death of a parent or spouse found “more intense grief reactions of somatic types, greater depression . . . anger and guilt with accompanying feelings of despair.” [158] Lewis and Lewis[159] identify “sudden” (guiltand mourning), “acute” (anger, overidealizing, fantasy), and “chronic” (remorse, relief, and guilt) reactions. The bereavement process continues in undulating waves, perhaps for as long as 3 to 5 years after a child's death. [160,161] and [162] No parents ever “get over” the death of their child.

The parents suffer from both the loss of the child and the loss of what the child represented to them. In our culture, children represent continuity of their parents' lives into the future, beyond death.[163] Children also are vessels into which parents tend to pour hopes and dreams not only for the child but also for themselves through the child's growth. Mothers and fathers differ from one another in their responses based on their own personalities, life experiences, and beliefs. The impact of the loss varies with the age of the child: A “different kind of pain” is associated with the death of younger and older children. [163] The older the child, the more experience the family has had with him or her. The more formed his or her personality, the greater the effects on the family system and its members, and the more extensive the memories.

A child's death may precipitate guilt in parents in reaction to feelings they perceive as negative toward the child. These usually involve wishes that “it would all finally end.”[159] When this kind of guilt is left unresolved, unexposed, and unexamined, it is a significant psychologic risk factor for the parent. Other special vulnerabilities involve the deceased child's role in the family. For example, the more emotionally dependent the family was on the child and the more the child was viewed by one or both parents as an emotional extension of self (in a need fulfillment or symbiotic sense), the more disruptive the child's death is to the family system. [142,164] The same is true for a child who served as the essential bond between parents or one who was the “communicator” for spouses in conflict.

Spinetta and colleagues[2] found that certain family coping efforts during the course of the illness can make a difference in their adaptation after the death. Better adjustment was seen in (a) parents who had a viable and ongoing “significant other” to turn to for help during the course of the illness; (b) those who had an open and responsive communication with the child during the illness and who gave their child the information and emotional support he or she needed; and (c) those who had a consistent philosophy of life that helped the family to accept the diagnosis and cope with its consequences. Participation in the care of the child during his or her life is associated with healthier bereavement responses. Similarly, attendance to the child during the dying process through home care or hospice care makes a significant difference, attenuating guilt feelings and anger.

Siblings should be informed by the parents of their dying sibling's status and of the child's death (if siblings are not present). This should be done in a way that is tailored to siblings' developmental stages. Siblings, like the parents, have immediate and long-term reactions. These reactions vary, based on factors that include the quality of their relationship with the deceased, whether they were same or different gender, whether there was a twin relationship, and whether the deceased was an older or younger child. Children of the same gender as the deceased sibling and twins are always at higher risk for failure in working through the loss. In the absence

of guidance, and depending on family dynamics, they may feel it necessary to take on the identity of the deceased.

Families generally do not seek professional help to deal with the upheaval and sadness of bereavement. If they were involved in a hospice, follow-up counseling is supposed to be offered. Parents, siblings, and grandparents benefit from the opportunity to reflect on and review the illness-dying-death experience until acceptance occurs.[17] When the atmosphere of the treatment site is accepting, families may return over a period of many years for spontaneous visits to the oncology service where their child was treated. Ongoing availability and interest in the health of these families should not stop at the point of the child's death. [1,17,122,165]

## SPECIAL PATIENT AND FAMILY CONSIDERATIONS

### Siblings

The effects of childhood cancer on the healthy siblings deserve special attention. Only in recent years have the special needs of siblings been recognized. Parents and the health care team must identify siblings' needs and intervene when appropriate. We must involve and educate siblings whenever possible, no longer viewing them solely as shadows of the sick child's affliction.[166] Support for the healthy siblings should occur at the time of diagnosis, throughout the course of the illness, and as part of bereavement support if the child patient should die. The strengths and vulnerabilities of each well sibling should be included in the initial family assessment. If at all possible, the well siblings should also attend the early family conferences. The siblings' early involvement minimizes feelings of isolation from the family and establishes an atmosphere of openness that sets the stage for communications among family members.[167]

Studies of the healthy siblings report predominantly adverse effects. Withdrawal, sleep disturbances, enuresis, crying, envy, guilt, preoccupation with their own health, somatic complaints, antisocial behavior, depression, fearfulness, separation anxieties, attention problems and poor school performance have been noted. [154,168,169,170] and [171] The reality is, unfortunately, that unless a crisis occurs, problems with siblings are rarely brought to the attention of the oncology staff. Our evaluations do not sufficiently emphasize the importance of siblings in the overall emotional management of the child. Obviously, their problems may appear temporary and less burdensome compared with the difficulties facing their ill brothers or sisters. [88,166] We now know they may even be lifelong.

Envy and rivalry among children exist in every family. In fact, hostile feelings among siblings are far more frequent in childhood than the professional literature indicates.[172] Healthy siblings often feel resentment because of the special care and parental attention given to the sick child. As a result, the healthy sibling may be fraught with jealousy, which sets the stage for experiencing guilt over escaping the affliction. This guilt may be further reinforced by the belief that the illness would not have occurred or would have had a better outcome if he or she had treated the sibling more kindly.

As the child proceeds throughout treatment, the healthy sibling often feels a sense of isolation and deprivation. Frequent hospitalizations result in the temporary loss of contact with the sibling as well as loss of contact with one or both parents. This creates an environment that is highly charged with emotion. [173] Often, healthy siblings share less of the parents' time and interest and may begin to question whether they still are loved. Most siblings attempt in one way or another to earn a status similar to the ill child. Early elementary age children may ask whether they can also “have some chemotherapy.” Most siblings complain of, and become concerned about, any aches and pains they develop. Some healthy children may wish they were the ones with the disease; others fear the day that they too will develop cancer. Lengthy hospitalizations or limited financial resources may cause siblings to be deprived of basic needs, including parental supervision. Emotional withdrawal or self-destructive acts serve as dramatic indications that the sibling is assuming more responsibility than is appropriate for his or her developmental level.[34] This may be more quietly evidenced by repeated excuses to be away from the home. An extreme example of self-destructive behavior and maladaptive coping is the well sibling who takes the sick child's medications or runs in front of a moving vehicle, for the purpose of being treated in the hospital. Siblings who engage in dangerous behavior or activities demonstrate serious difficulty in coping with the current home and medical situation. In such situations, intervention by an experienced mental health professional is essential. This intervention should be introduced and encouraged by the physician or members of the health care team who have the family's trust and confidence.

Feelings of isolation are exacerbated for siblings if the family moves to live closer to the treatment facility. The healthy sibling experiences a loss of community, friends, and playmates, and needs to adapt to the changes and stress of attending a new school. Although siblings often feel neglected when parental attention is focused on the ill child (and upset by having their life circumstances altered), most fear confronting their parents with negative feelings. [174] This results in an increased sense of isolation from their family and at times a sense of personal failure. Oncology staff and other health care professionals can assist families considerably by directing their attention to the needs of their well children and by encouraging open communication among all family members.

Several other measures can be helpful in easing the emotional stress on siblings. The parents or treatment team need to discuss the ill child's diagnosis, treatment, and prognosis with siblings at a level they can easily understand. [175] Siblings need to be prepared for the physical changes that their brother or sister will undergo and for the possible role realignments in the family. Siblings must believe that their thoughts, concerns, and questions are important and acceptable. This includes feelings of anger toward their parents and jealousy or hostility toward the sick child. Siblings need to be reassured that they will be kept up-to-date on their brother's or sister's treatment progress and, whenever possible, included in their care and management. (If the treatment site is in a community distant from the home a free site on the Internet, http//:www.caringbridge.com, makes keeping up with progress and problems easier.) When treatment requires parental absence from home, a regularly scheduled time should be arranged for the parents and siblings and ill child to talk by telephone. This helps to lessen separation anxiety and provides the sibling with a sense of belonging and contact as well as inclusion in the sick child's care.

Few investigations have reported children's reactions to the death of a sibling from cancer. Spinetta and coworkers [2] found that siblings' symptoms or unresolved feelings persisted in most families, including crying spells, health fears, feelings of remorse and guilt, and refusal to discuss the deceased child even 2 or 3 years after the child's death. In a study based on parental report, Lewis [165] reported that more than half of the siblings required some sort of medical consultation after the death of the affected child. In a study based on psychiatric patients, Cain and colleagues [176] found that the surviving children had a heightened awareness and fear of death, believing that it could strike someone close to them at any time or themselves when they reached the same age as the dead sibling. All authors agreed that the experience of a child's death has a profound effect on the siblings. The factors that determine a child's immediate and long-term reactions to the death of a sibling are multiple and include the following: (a) the level of communication with the sibling during his or her life; (b) the parents' explanation of the diagnosis, treatment, and prognosis to the surviving child; (c) the child's ability to express both positive and negative feelings about the sibling and the disease and hospitalizations; (d) the age, sex, and developmental level of the surviving sibling; (e) the child's preexisting relationship with the sibling and parents; (f) the parents' reactions (expressed thoughts and behaviors) to the death and their subsequent attitude toward their remaining children.

### Marital Disharmony and Divorce

The crisis evoked by childhood cancer taxes every marriage. Parents who have supported each other through previous crises, who can share with each other expressions of sadness, anger, frustration, and hope, who are able to make their child's illness a priority, often eventually find their relationship strengthened. For others, the stress of a child's cancer exacerbates previous marital problems, especially those of long duration. The child's parents may appear to be emotionally distant, coping with the situation in isolation from each other or using it to fight their unresolved battles. Such marital disharmony negatively affects the entire family's emotional adjustment to the disease and requires mental health intervention. [177]

If the child's parents are separated or divorced, special efforts need to be made by the health care team to keep both parents informed about the child's diagnosis, treatments, and progress. Parents maintaining a friendship after separation or divorce tend to have a less intense experience of loss. [178] They also tend to find the stresses associated with the disease and treatment less severe than do those parents who continue to have difficulty communicating or being in the same place together. The latter situation often presents a dilemma for staff. In an attempt to gain a sense of control and feel included in the child's care, some parents vie for alignments with certain staff members. The staff must not become enmeshed in the family system by splitting their alliances between parents. In such cases, the child often is caught in the middle and is left feeling guilty, alone, and without family or staff support. The health care team also needs to be aware of custody decisions, parental visitation arrangements, and possible remarriage and stepfamily relationships.

### Single Parents

A major problem for most single-parent families is task overload.[178] Single parents often find themselves in a situation of financial hardship. Struggling to work, provide childcare, maintain a home, have a personal life, and possibly deal with visitation arrangements can be most difficult. When a child develops a life-threatening or chronic illness, single parents feel especially isolated, alone, and without an adequate support system to meet the crisis. They are often without another person with whom they can share the responsibility of the sick child's care or the daily decisions that need to be made. After diagnosis and throughout the treatment process,

single parents often describe themselves as feeling overwhelmed, incompetent, indecisive, guilty, and sad. They may also experience considerable anger toward former mates, who may provide little if any emotional or financial assistance, or even toward other families who seem to have “fewer problems” than they do.

The health care team can assist single-parent families by identifying the economic, psychologic, social, and support resource needs early in the child's treatment. Each family member's strengths and weaknesses must be assessed. Parents need to be encouraged to turn to staff, extended families, or other nonfamily supports for help in decision making, reassignment of home responsibilities, and financial assistance (especially travel and child care costs) when needed. Individual and family counseling often helps single parents find the strength needed to cope with the daily demands and often overwhelming stress with which they are confronted. With caring and sensitive intervention, these families may discover new resources within themselves, within their families, and with their extended or nonfamily supports that can be used again through the course of the child's illness. [179]

**Preexisting Psychopathology**

Preexisting psychopathology in any family member adds to the challenge of dealing with a chronic, life-threatening condition. Preexisting major conditions (anxiety and phobic disorders, developmental disorders including severe learning disabilities and severe attention deficit disorder or attention deficit/hyperactivity disorder, dementia, severe personality disorders, mood disorders, the schizophrenias, substance abuse) are a significant drain on the family system. They present a potential disruption of prescribed treatments for the cancer patient. Any one of these disorders can deplete a family's time, exhaust its emotional resources, and drain it financially. The consequences are different if one of the parents is ill than if a sibling or grandparent is affected. Obtaining a history of problems before the onset of the child cancer patient's illness is essential orienting information for the health care team. Note that even dramatic psychiatric symptoms can be reactive, at least in part, to the stresses and disruptions of the cancer diagnosis and treatment course. When the behaviors of family members present patterns that raise questions about psychopathology, it is wise to seek a consultation with an experienced social worker, psychologist, or psychiatrist.

**Cultural Differences**

When considering family vulnerability and crisis, the effects of cultural attitudes are of increasing importance. This is especially relevant in countries such as the United States, Canada, and those of Western Europe and Great Britain, in which there are significant immigrant populations. [180] Cultural attitudes are outgrowths of value orientations. They vary from one culture to another. [181] Clinicians must move beyond the limitations of traditional approaches, learning how to determine the impact of cultural differences, poverty, discrimination, and acculturation issues. [182] Such differences affect how each family perceives and responds to illness, treatment, and death. For example, different cultures have different expectations of the medical system, different beliefs and attitudes about patient care and disease causation, and different attitudes about death and rituals around death. The effects of cultural barriers on communication have a major impact on both the family's reactions to their child's illness and their ability to place trust in the health care team. [183]

Misunderstanding, confusion, and alienation often result from the failure of the medical team to consider sociocultural factors in the child's care. Learning about the beliefs, attitudes, and behaviors of the family's ethnic group enhances the therapeutic alliance. Explore with the family their understanding of the illness, their expectations of the staff and of the treatment offered, and the role of religion in their daily lives. The health care team ideally will identify whether (a) conflict exists between religious beliefs and treatment decisions; (b) the parents can meet their child's need for information; (c) problems exist because of language differences or difficulty using the supports available within the medical environment or community; (d) parents are able to accept assistance from others whose lifestyles differ from their own; and (e) parents respond better to informal or formal interactions with staff. Families are often eager to share with staff information about the family structure, culture, roles within the family, and belief systems when an interest is expressed. But the health professional must create the interpersonal atmosphere that signals to them an interest, a caring about learning this information. Caution must be used to determine that words as well as images used have the same meaning to the family as they do to the professional. Once this information has been elicited, it may be possible to mobilize available sources of support that could further reduce the stresses of being in a different environment and assist in the family's adjustment. If the family does not speak English, regularly scheduled meetings with the family are essential. These meetings should include the child's physicians, nurse, and social worker, as well as a staff member or reliable volunteer who speaks the language, understands the culture, and is trusted by the family. Such meetings avoid communication breakdown and enhance the quality of patient-family-staff relations.

**Completion of Therapy**

The completion of therapy is another point of extreme stress. Discontinuation of treatment encourages an increased sense of hope for extended survival. Yet this hope is clouded by anxiety from several sources. First, the child and family can no longer cling to the routine of taking medication to maintain security and optimism. Parents often believe that they are not actively fighting the disease and that relapse is therefore more likely. Families also fear a loss of contact with the treatment team.

Families require extra support and education at this junction. Physicians must outline reasons for discontinuation of therapy, the possibility of relapse with or without treatment, and the risks of continuing therapy longer than necessary. [184] Treatment options in the event of a relapse should be explained to the parents. Families can also be reassured by the knowledge that the child is still a patient and will be monitored closely. [31] They may, at this point, find it useful to increase their use of available communication resources, including chat rooms, through the Internet ( Table 46-4).

**Long-Term Survivors**

The anxiety felt when therapy is completed dissipates slowly as months pass and the child remains free of disease. [185] With the diminishing of concerns about disease recurrence, other worries take their place. These include fears about the long-term sequelae of the illness and treatment. Long-term survivors of childhood cancer face future physical and psychosocial risks, many of which are just beginning to be described. The survivors and their families must learn to live with uncertainty about disease recurrence and future well-being to a degree not experienced by most others in our culture.

The survivor must first cope with physical sequelae. These can range from changes in appearance (e.g., obesity, physical disabilities left by surgery) to defects in major organ systems (e.g., cardiac, liver, renal) to fertility problems to the risk of second malignant neoplasms. [186,187,188,189,190,191,192,193,194,195,196,197,198,199,200,201,202,203] and [204] Routine follow-up of the long-term cancer survivor should include screening for these potential late effects as well as age-appropriate education regarding special lifelong health risks and necessary health maintenance practices.

Potential disruptive effects of childhood cancer and its treatment extend into other areas of the survivor's life. Developmental disruptions experienced during treatment have undeniable implications for future psychosocial adjustment. The degree to which these disruptions affect the child's later adjustment varies. Fewer subsequent adjustment problems are seen with increasing time since disease onset and younger age at diagnosis. [72] Older children and adolescents may be particularly sensitive to interruptions in their developing peer and intimate relationships, school and extracurricular activities, and plans for future lifestyle and occupation. [204] They are more likely to exhibit agitation, restlessness, and hyperactivity. [200] Reports of psychosocial adjustment problems are conflicting. Descriptions of high rates of psychiatric symptoms [72,205,206] contrast with hypotheses that overall adjustment is unaffected by the experience of childhood cancer. [191,200,207] The dichotomy may be that these children are not at greater risk for serious psychopathology than healthy children. Rather, they may be more vulnerable to intermittent and minor adjustment difficulties [208] as well as problems in family cohesion. [209]

Specific educational and occupational achievements and choices may be affected by cancer survival. The academic ability of the long-term survivor may potentially be affected by intellectual deficits [186,210,211,212] and [213] and learning problems. Cancer therapy, specifically cranial irradiation, has been associated with problems in attention and concentration, performance under pressure, visual and auditory memory, and mathematics skills. Language skills appear relatively unaffected. [79,214,215,216,217] and [218] These potential learning problems, coupled with disruptions in school attendance, may limit the child's educational achievement and occupational attainment. [219]

Recommendations for intervention include baseline assessment and periodic monitoring of neuropsychologic functioning. Continuous evaluation of academic performance as a part of regular aftercare permits prompt identification of learning disabilities that may not appear for several years. Once identified, learning problems can be dealt with through an educational program tailored to the individual child's specific areas of strength and weakness.

Even when they are successful in overcoming learning and educational barriers, long-term cancer survivors may encounter difficulties in the community and

workplace as a result of their cancer history. Hiring discrimination, ineligibility for health and life insurance, and employers' attitudes about cancer may all complicate the cancer survivor's entry into the work force.[79,218,220]

### Cost

Chapter 52 discusses financial issues in pediatric cancer care. Few families undergo the rigors of years of cancer treatment and follow-up without considerable economic stress. Early assessment of the family's financial situation is essential to lessen current and future economic stress on the family. Many families find it helpful to keep a record of all incurred expenses.

The cost of treatment can be divided into direct medical charges and nonmedical out-of-pocket expenses. Nonmedical costs, including extra food and clothing, transportation, long-distance telephone calls to doctors and family members, meals, temporary housing near the hospital, care for siblings, and miscellaneous items, have a greater immediate impact on the family's budget. Two independent surveys of these expenditures, one in Kansas and the other in England, have reported virtually identical results.[221,222] Approximately half the families indicated that out-of-pocket expenses plus loss of pay amounted to at least 26% of their weekly family budgets. Four factors influenced nonmedical expenses. For obvious reasons, hospitalization (as opposed to outpatient treatment or no contact) and distance from the treatment center were associated with higher expenses. As the child's ability to engage in normal activities deteriorated, expenses increased. Costs also increased with family size. The more children in the family, the greater the expense of caring for them in the parents' absence. Single-parent families also had considerable expense, presumably related to child care for siblings when the patient was treated at a medical center in another community.

Direct medical costs for cancer treatment are prohibitive. Outpatient medical costs vary with diagnosis, with a large spread between cancers requiring intensive treatment and those involving only routine follow-up. In families whose children eventually died of their illness, mean annual medical costs amounted to almost twice the mean annual income. The diagnostic and terminal stages of illness accounted for more than 50% of these charges. The major source of payment of these bills was insurance or the health maintenance organization. Nevertheless, outstanding debts to the cancer center as long as 3 years after the child's death were frequent.[223,224]

Half the families experience costs related to their child's illness that amount to at least one-third of their monthly income, more than the level described as “catastrophic.”[225] This total financial burden consists of medical charges not covered by third-party carriers, nonmedical out-of-pocket costs, and loss of pay.

Economic impact adds significantly to the family's overall distress. Even when the financial hardship is less extreme, it has long-lasting deleterious effects on all family members because of the depletion of resources over an extended period. Parents and siblings have fewer needs met because such a large proportion of the family budget goes toward the care of the sick child. There are no simple solutions for the financial plight of these families. Early assessment of socioeconomic vulnerability may include evaluation of insurance coverage, the availability of community resources, and job-related issues.[47] At best, such an assessment sketches the support needed.[226]

## FINAL WORD

Despite the life-disrupting and life-threatening nature of childhood cancer, most families display remarkable resilience in adaptation.[227] Working to mobilize the strengths of families adds enormously to the effectiveness of the oncology treatment team. An attitude on the part of the physicians and nurses that child and parents (to the extent possible in each situation) be included as members of the treatment team is essential. Even multiproblem families have strengths that can be tapped and mobilized by the team. Ignoring at the outset signs of significant vulnerabilities can wreak havoc with the most brilliant treatment protocols. Because oncologic diseases are chronic processes, comprehensive psychosocial care begins with early assessment of family strengths and vulnerabilities. This care continues throughout and beyond the course of the disease.

An ongoing multidisciplinary approach to the psychosocial care of children and adolescents and their families is basic to responsible modern treatment. Interventions and strategies aimed at identifying the continuum of coping responses, building on family strengths, assisting families with special needs, and enhancing adaptive coping skills are essential to facilitating both family growth and survival through the crises generated by childhood cancer. One hopes that the economics of “managed care” in the United States and countries around the globe will allow for incorporation of this concept into all oncology programs.

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# THE OTHER SIDE OF THE BED: WHAT CAREGIVERS CAN LEARN FROM LISTENING TO PATIENTS AND THEIR FAMILIES

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## INTRODUCTION

The pediatric oncology literature abounds with studies, many complete with impressively significant *p* values, confirming what common sense dictates—that attention to the emotional needs of the afflicted child and family is an essential component of the comprehensive care of a child with cancer. The optimum practice of pediatric oncology requires far more than the knowledge of a particular cancer and its treatment. In many ways, the formulation of a diagnosis and treatment plan is the easy part. Often more difficult and arduous is the delivery of care to a family whose stability is threatened by the potential loss of a child. Conflicted by fear and occasionally by guilt, the family members also have to overcome a sense of loss—they are now marked by the diagnosis of cancer, which will forever affect the fabric of the family life. In this setting, the pediatric oncologist must be not only a diagnostician and coordinator of treatment but also an educator, a friend, and a constant source of support.

Cancer has now become a chronic disease for the majority of pediatric oncology patients, replete with long-term effects of therapy even after cure. The messages and needs directed to all medical personnel by the children and their families, then, become particularly important if we are to achieve a “truly cured child.” From time to time, just as they are required to participate in continuing educational programs, physicians and medical personnel involved in the care of children with cancer need a gentle reminder of the problems facing the family and patient. In this chapter, we examine the treatment of the child with cancer from the perspective of the patient and family members. Some recurring themes expressed by patients and their families are described that shed light on the other side of the hospital bed. These themes, although at times self-evident, are important reminders that to the family, every conversation or event may be carefully analyzed for its significance. Therefore, along with the requisite medical competency, the pediatric oncologist has the added responsibility of maintaining the highest level of personal integrity in his or her relationship with patients and their families.

The training of a pediatric oncologist concentrates on mastering the facts of diagnosis, epidemiology, statistics, treatment, and prognosis. Such information can be both wonderful and daunting—wonderful because it provides the physician with the power to heal the cancer patient, daunting because one has to decide what information to dispense and when. The course of disease in a particular patient is predicted in general terms, based on statistical considerations. Statistics are helpful in portraying the natural history of a specific cancer and its expected response to therapy, but to the patient, treatment either succeeds or fails. Although a physician may have treated a particular cancer many times before, for the patient it is the first and only time that matters. The chilling reality is that for each child undergoing treatment, it is either 0% or 100% successful. The difficult task, then, is to guide the patient and family through a course of therapy when the outcome is uncertain and when, indeed, the fear of failure hangs over the outcome. This requires a delicate balance between fostering hope while maintaining a realistic perspective of the likelihood of survival with or without long-term effects.

The chapter is organized into two sections. The first section on general issues of communication and trust addresses a variety of topics concerning the nature of the relationship between care provider and the patient/family unit. The second section on major events in the care of children with cancer addresses the calendar of treatment, moving through some of the milestones of therapy and afterward. We offer no absolute solutions; rather, we present comments on issues deemed important by families and patients. The statements in extracts are actual comments of patients, family members, friends, colleagues, and some of us. They are intended to illustrate key issues, which are subsequently discussed in the text. Although many of the quotations and comments are targeted to the pediatric oncologist, they are applicable to the wider community of caregivers. It is our hope that all caregivers will become more sensitive to the predicament of the individual patient and his or her family, which will contribute to a better outcome for the patient.

## GENERAL ISSUES OF COMMUNICATION AND TRUST

### Loss of Control

> “It was such a blow. I never realized my son might have cancer when they sent me to see a pediatric oncologist. I didn't know what a pediatric oncologist did. I'd never heard of the word before.”

> “When the doctor came into the room and told us my son had leukemia, I was shocked. I had no idea. The first thing I did was run to the bathroom, throw up, and then cry. When I walked back to the room, I knew my life had changed. It wasn't going to be me telling him what to do. Someone else was going to tell us how to live and what to do. I was so scared and numb inside. I didn't know what to do for days. I barely ate or slept and stayed at my son's bedside.”

Perhaps the most difficult issue that families must face is the loss of control over their own lives. In particular, this affects not only the organization of a daily routine but also the perception of the family unit. The shock and disbelief forces the family to confront its existence. Many parents report that they lose a sense of “unlimited potential.” The possibility that the child could die before the parent shakes the foundation of a family, whose purpose is to lovingly raise the next generation. It is this loss of a “normal” future that disrupts the stability of a family. Family members fear that the structure of the family may unravel. They fear that they may fail to do what is necessary to preserve their own lineage. The sanctity of a family structure will never be without doubt. Will the child die? Will he or she return to school? Will he or she be able to become a parent? If remission is achieved, will the disease recur?

> “After my daughter had been in the hospital for 3 weeks, I didn't know up from down. Everything was a blur. Friends and family came forward to take care of things

> for us, but it wasn't until the nurses suggested that I go home and see my other kids and the dog that I realized what I had to rebuild. At first I thought I never would, but the staff kept encouraging me."

The disruption of a family's life assumes many forms. Aside from the temporal, social, and often economic upheaval that accompanies the treatment and care of the child, the uncertainty of the future has a profound effect on the daily routine. Initially, the family unit is paralyzed and all attention is on the afflicted child. For the rest of the family, even scheduled activities such as gathering at meals or bedtime rituals may be disrupted. This frequently leads to an additional strain, particularly in siblings who may not fully understand the changes in family activities. Although their resentment is understandable, addressing it in a constructive manner can be difficult. Siblings may be confused by feelings of jealousy and guilt, resulting in a sense of being trapped in a no-win situation. They may be forewarned of changes in routine, but they are still frightened and upset. Their frustration and resentment may surface at the most inopportune times. Parents are not as available to participate in normal activities nor can they be as attentive as usual to the needs of other family members.

**Misconceptions about Illness and Treatment**

> "My sister died of ovarian cancer 8 weeks before the diagnosis of my son. Although her prognosis was very poor, an aggressive form of chemotherapy was begun. She suffered terrible side effects for 6 weeks before her death. When I learned my child had leukemia and listened to the details of the protocol, I couldn't believe that it would "work." All I could imagine was that the treatment would prolong his suffering, and he would die anyway, perhaps 8 weeks later."

Compounding the anxiety and confusion are the fantasies and often incorrect ideas formulated by the family. Many of these arise from a misunderstanding of the specifics of a treatment program or from facts gleaned from the lay press, Internet sources, an out-of-date medical text, or friends and family. Ideas may also be influenced by previous experiences with cancer. These can inhibit a family's ability to organize its priorities, partly because of the uncertain future and partly because of the necessity of the surrender of control to the medical establishment. Some families describe the first weeks as a "waking nightmare," and for each family the nightmare is different. The unique constellation of previous experiences and personalities in each family gives rise to a wide spectrum of coping abilities. In this regard, it is important to consider the family's cultural, religious, and ethnic background when addressing the issues of preserving both the daily routine and the dignity of family life.

Encouraging a family to set priorities to reestablish the daily routine has pleiotropic effects. Affirmation of a life outside the hospital or clinic comes as a relief to the family, helping them to recognize the value of life outside the confines of medical care. It also affirms the need to reestablish an existence after the shock of the diagnosis. Because so much importance is attached to recommendations of the care-providers, signals from them to attend to other phases of the family structure releases the family from concentrating only on the affected child. Families may need to be warned that even if they are doing everything possible to accommodate the siblings, it may not be enough from the siblings' perspective.

Underlying the rational concerns of the child's health is an irrational fear of never recapturing control of the family unit. In some circumstances, it may be prudent to state directly that the care provided by the medical team is but a small contribution to the treatment of the child. The difficult task is to allow the family to attain a sense of independence and control while the pediatric oncologist continues to direct the technical and medical aspects of the child's care.

**Styles of Communication**

***Trust***

> "Every time the doctor sat down with us, she always made a point of talking to all of us—my husband, my son, and me. We knew we could count on her. It seems that every time I had a question, she would repeat it back before she answered it, sometimes for longer than I wanted, but the answer was always honest. I liked the fact that she never glossed over my questions."

> "When the doctor, without a smile, found me in the play area, I knew something was up. He asked me to come in and sit down. Then, my worst fear was right. My son did, indeed, have cancer."

Parents and patients must have the impression that the physician is truly focused on their needs. They must believe that they can ask questions before the physician rushes off. Sitting down and directing your full intellectual and visual attention to the family and patient is a strong statement of commitment. Serious or lengthy discussions should not take place standing in a hallway or waiting room, and bad news should never be delivered in this way. By sitting down with family members, the physician demonstrates that only they matter, while everything else is on hold. Of course, this is good patient care, but the serious nature of treatment makes this especially important.

Direct physical contact, whether it be shaking hands, touching a shoulder, or giving a hug also carries great comfort. Tactile communication breaks down a common interpersonal barrier and is a demonstration of one's earnest concern. Parents and patients describe it as an almost "magical connection," interpreted as a sign of compassion.

> "It was always hard to recall everything the doctor said. Whenever I took my son to oncology clinic without my husband, the second I got home I was subjected to the third degree. Every detail had to be recounted and then interpreted. Sometimes it was hard to reconstruct every point for my husband. Every word was analyzed closely for its hidden meaning. There were times I wish I had a tape recorder just so he could hear everything the doctor said."

The physician does not speak only to those present in the room or on the end of a telephone. Families turn within or to friends for clarification or solace. The words of a physician will be repeated over and over, sometimes changing with each version. This necessitates clarity in explanations or instructions. Do not be annoyed by restatement or repetition, because this is often an attempt by the family and patient to make certain that they understand. If the family or patient understands the issues, the subsequent explanation to others may be easier. It is not uncommon for parents to argue or get upset over the discussion or recounting of an earlier meeting. Because so much hangs on the physician's words, choosing them discreetly and appropriately for the individual family is necessary. When one parent visits, particularly if there is a divorce or separation, it may be prudent to offer the opportunity to speak with the other parent separately or on the telephone to diffuse the tension between them.

Trust in the physician also requires that the physician be honest in shortcomings of knowledge and ability. If the answer to a specific question is unknown, then say so. If it is possible to seek an answer and return with it, then let the family know of the plan. When an answer is not available, inform the patient and family; in some circumstances, it may be prudent to point out that it is beyond the pediatric oncologist's ability to obtain the answer immediately—for instance, when waiting for a consultant's opinion or the results of a test sent to another center. In any circumstance, if the physician does not follow through, his or her credibility may be undermined. When an answer is impossible—such as to the question, Will my child live, for sure?—be emphatic about not being able to answer the question satisfactorily. Explain that there are no guarantees and that every patient is different in the expression of his or her disease. In addition, it may be prudent to emphasize that each patient has his or her own individual care plan.

> "Hope was the hardest thing to keep up. One minute a doctor scared us with bad news, or at least, that's how I saw it—reading between the lines, of course. The next minute, a nurse would reassure us that everything was okay. I often felt like a ping-pong ball, back and forth. In the end, it was hope that sustained me. It let me stay up every night at my child's side, even when I was exhausted. If that were gone, I don't know how I could have survived some of the bad nights in the hospital or the long nights worrying at home."

It is a daunting task to strike the right balance between the truth of pediatric cancer prognosis and the sustenance of hope, even in the face of the imminent demise of a patient. What is inadvisable is to crush hope—even in the preterminal phase. The pain and suffering of failed therapy will surface over time, but the deliberate (or not so deliberate) suppression of hope takes away a critical element of both individuality and a motivation to keep going—whether it be on protocol or in supportive care.

It is important to define hope according to the circumstances. Although each family seeks the truth concerning the medical status of the child, they strongly desire a successful resolution. Remember that hope can vary from a mundane point, such as fitting someone in for an appointment, to relief from pain or achieving long-term cure. Sometimes hope is hospice care or help from a religious leader in preparing for the end of a child's life. Providing hope can also mean being helpful and supportive after one has exhausted current medical expertise.

### *Questions*

> “I used to get so mad because the doctor always cut me off before I finished my question. It seemed he was always in a hurry when I wanted to talk about my concerns. We had time for his agenda, but he did not have enough time for mine. Thank God for the nurses; they listened and answered my questions.”

During any discussion, listen to the complete question. Many parents and patients are frustrated when an answer begins before the question is finished. The process of asking a question can be very therapeutic. Particularly when the question is difficult (for example, one that pertains to death), remember that it may be the accumulation of hours or days of worrying. Both the formulation and process of asking the question may also be the first admission of a problem, one that may have been denied or previously not appreciated. Furthermore, it may also require special patience to wait for the articulation of the problem or question, especially when the parents are not particularly articulate or are affected by emotion or stress or there is a language barrier.

Reassure the family and patient that they can ask questions at any time. This is especially important during the initial discussions of diagnosis, prognosis, and treatment. Parents and patients should be explicitly informed that important information or instructions may be repeatedly stated by the staff. Reassure them that this is not a judgment on their intelligence but, instead, a way of fully educating the family and patient. In the same way, it is important to encourage them to ask again if they do not understand a point or problem.

It is difficult not to lose one's patience when having to repeat oneself; it is easy to be irritated when a conversation appears to be repetitive; but repetition is often focused on facets of treatment or illness about which the parents are most anxious. Parents will be better educated, more compliant, and appropriately inquisitive if they are “permitted to ask” with no fear of being inappropriate or taxing the patience of the caregiver.

Particularly at the end of a difficult conversation, allow time for further questions. It may be prudent to end a discussion with the question, “Do you have any other questions?” and wait an appropriate time before concluding the conversation. The extra time of silence may lead to a discussion of “taboo subjects,” such as death or relapse, issues that clearly are of concern but are rarely articulated. It often takes time to summon up the courage to ask difficult questions. Some families need the extra encouragement, with either nonverbal or verbal cues, that enables them to venture forth with a painful query.

## Educate the Patient

> “When I was on the ward, I once watched one little girl get moved around like a Raggedy Ann doll. She did everything she was told to but was afraid to try anything new. One time I suggested we do something that the nurses wouldn't like. When she did it she was so proud to have shown them up. Afterward, she said that she was getting back at the cancer for trying to hurt her. She believed that she had cancer because she did something wrong. When she realized she could get into trouble and not get sicker, she started to act like all the other kids. She even had fun with us.”

Even for children, the diagnosis of cancer is overlaid with interpretation. In this regard, children are no different than adults. Each child attaches a unique meaning to the illness and creates a model within which the experience is viewed. For example, a child may believe that he or she is being punished for a previous mistake, such as hurting a sibling or breaking an object. Others may see the disease as a punishment that is deserved. The difficult task for the physician is to extract these perceptions to dispel such myths and to use them as a framework for better communication. Furthermore, the physician who assumes the difficult task of correcting these perceptions must do so in a way that is sensitive to the child's ability to understand. Although some children regress during therapy, most children who have cancer mature quickly because of the responsibilities of the illness. Many times, the child becomes the strongest member of the family, calming even their parents. Children become quite knowledgeable of the implications of their disease. Thus, speaking to a 6-year-old as to a 10-year-old may be appropriate in many instances.

> “There were some great doctors who took care of my child, and then there were others. The good ones played with kids. They had fun and made them laugh. If they could play, then my child trusted them. She knew when to believe what the doctor said. If a test was painful, she preferred to have the trusted doctors perform it. The trusted ones were the ones who won her affection in small ways. The disliked doctors were the ones who talked over her or were condescending. They didn't make my child feel special.”

Talking to a child with cancer requires sensitivity to the maturity of the child as well as a working knowledge of the defenses children use at the time of crisis. Children are remarkably apt at identifying honesty and directness. They respond to those who are interested and, in particular, to those who take the time to include them and make them feel special, even if it is at a time of pain or discomfort.

When a physician speaks to a child, he or she is being observed by the parent. Many of the issues raised elsewhere are quickly analyzed by the parent during the discussion. Afterward, the parents will take cues or hints from the patient as to how the physician communicated with the child or adolescent. To have the parent as an ally for therapy, the child has to have faith in the physician.

Even after the initial discussion, children and adolescents may deny their diagnosis. They may actively believe it is not true and refuse to accept the changes in lifestyle. Others may act out and challenge the authority of a parent or of a medical recommendation. It is necessary to be sensitive to how much a child denies, particularly when a seemingly obvious and direct explanation does not result in a clear understanding of a recommendation or situation. Throughout therapy, denial represents one of the most difficult obstacles for the physician in establishing and maintaining the trust of a patient.

In talking to pediatric and adolescent patients, a clear explanation of the reason as well as the specifics of what is to happen will greatly enhance cooperation. Fear exists in the unknown. A seemingly noninvasive test, such as a magnetic resonance imaging scan, can evoke panic and distress in a timid or claustrophobic child. If a child has sense of what to expect, a large burden of fear is removed.

A physician can only say so much to a child or adolescent in any one discussion. Remember that these patients are scared of what is being said. With younger patients (e.g., patients younger than 6 years), the parent is often the best communicator of news or recommendations. The adolescent, who may be estranged from a parent, presents an even more formidable challenge. In this circumstance, it is particularly important to recognize both the maturity and the independence of the adolescent. This may require private conversation without the presence of a parent, although never to the long-term exclusion of the parent. Often, the parent needs to be reminded that the adolescent requires a level of independence and must share in the decision-making process so as to have as much control over his or her life as possible. Children of all ages can see the physician as an alternative authority figure to family members. Thus, it is possible, with the assent of the family, that the medical staff can help the parent with the child's behavior and education. Talking independently with the family members can also influence the child's relationship with the family during a time of great stress and confusion.

### *Listen to the Patient*

> “One of the other patients on the ward had his leg amputated. He had crutches but had little use for them because everyone wanted to help him. He would get so frustrated when everyone tried to do something for him. One day, he was allowed to get out of bed and walk out of the room by himself. It was real hard, but he was proud. He worked hard to overcome this, and 2 years later he could beat me or almost anyone else in a foot race.”

It is important to listen to the needs of patients. What may seem trivial or minor in assistance given may actually represent a significant achievement. Realizing that some patients have to overcome obstacles in their own way and on their own schedule requires a sensitive eye for rebuilding self-esteem. Help is always appreciated when it is appropriate but not when it is assumed. It takes great insight to see that the greatest help may be to only watch and support. The consequences of illness remain with the patient for a long time, and being able to perform the ordinary may instill pride and self-worth. Many patients advise, “Don't say you can't do it; instead, say you can't do it, yet.”

The limitation of activities should be presented in a sensitive and optimistic manner. Give the patient the opportunity to overcome the problem. Children prefer a challenge over a statement of fact, which, in some cases, may lead to an unconscious fulfillment of the prophecy. Patients understand that limitations exist but resent the announcement of an absolute. For example, in warning about vincristine toxicity, it is frightening for the patient to hear the physician say, “You will become uncoordinated.” But the physician may offer the possibility of overcoming the problem by explaining, “You may lose some of your reflexes and may not play some games as well as you used to, but that doesn't imply that you can't do the things you like to do.”

Patients of all ages appreciate the belief that they may succeed. In the case of most children and adolescents, they usually believe that they will be one of the “lucky”

ones and survive. Their focus is on achieving this goal, but the manner in which they conduct themselves may vary greatly. They need the support of those around them to feel that they are in control of their lives and that the disease has not robbed them of their potential. Children want to know, either overtly or subconsciously, that they are mastering their illness and not being mastered by it.

> “It is hard to explain, but you think of strange things when you're sick and lying in a hospital bed. There was one time when I was real sick with high fevers and all I could think about was whether I could be a mommy someday. Now talk about getting confused. The doctors and nurses were completely stunned when I asked about this. They said, “Don't worry about this, now. We'll talk about this later.” To me, it was and always will be a big question. I just wish they didn't blow me off.”

One of the hardest lessons that providers have to remember is that each patient responds to the crisis at hand differently. The appropriateness of remarks and questions are sometimes hard to gauge, but usually, on further exploration, the underlying reason is understandable. In particular, children may appear to be more concrete in their stated concerns and, on occasion, confused as to the appropriateness of a thought or feeling. In response to this, each question or comment deserves the benefit of an answer. Reviewing the question or comment many times reveals important issues or fears may be evident and, in the course of the discussion, dispelled. Such comments or questions, however, may lead to exploration of issues that many find difficult to discuss, such as fertility or death.

> “Living with cancer is like having a roommate that you can never get rid of.”

For patients, the disease never goes away. Children with cancer will undoubtedly experience nonmedical repercussions for years to come, in the form of discrimination or unwanted, special attention. They will spend the rest of their lives explaining the type of cancer and its treatment. This presents a perpetual dilemma of what to tell others. How much do they tell friends about why they may look different or be restricted in activities? In a sense, the survivor is marked by all who know, primarily because they are seen as different. When can they assume that others are comfortable with the changes in their life? It is critical to listen to these concerns, even though they may appear to be mundane. Patients appreciate the support and understanding of the medical staff, because their approval may help to rebuild self-confidence and self-worth.

Constantly, survivors of childhood cancer are faced with the challenge of continually explaining what has happened. It is difficult to appreciate the nature of the burden of disclosing such an intimate yet terrifying story to friends and strangers alike. Whether it is warranted, as in an application for insurance or schooling, or whether it is a consequence of curiosity, either stated or implied, on the part of others, the survivor can never get away from the events of the past. In this regard, the medical staff offers a unique ear in listening to families and in turn can offer suggestions about how to face the public at large. Whether they overtly state it or not, families tend to develop a sense of being different. They are the “unlucky” ones who have been “hit by lightning.” Furthermore, many believe that “if it has happened once, why can't it happen again?” The lingering fear that the cancer may recur never goes away. Having to explain what has happened takes a toll. Although in some circumstances the recounting can be therapeutic, in others it may be a source of embarrassment or frustration. The diagnosis never fades, even though there may be no further evidence of disease. When a patient completes therapy, it may be necessary to address these issues, often providing a clear description of the events of the past. By offering a summation of the events, the words and phrases may be helpful in future conversations in which the patient will repeatedly engage. If the patient and the family are given the opportunity to discuss how others view them, it may be possible to explore the kinds of support necessary to further rebuild self-esteem. Support from the medical staff in the form of acknowledgment of the changes that have taken place both medically and personally is crucial to ensure a smooth transition from patient to survivor.

> “When a nurse asked me what I wanted to do when I was discharged, I tried to explain that my aspirations were more than getting discharged. Patients want to do what every ordinary person does. They want to look like everyone else. They worry about the same problems as others, but in addition, they have the burden of illness. Things are different because they are sick. Still, they worry about whether they can go out in public with a low white blood cell count. Can they go to school or a team practice? How will their friends react to the change? What will they think of hair loss? How much can they sit in the sun, even though radiation therapy is not completed? Will therapy conflict with attending a game or birthday party? After a while, treatment becomes routine, but with an underlying fear that something may happen that is out of the ordinary and will disrupt future plans.”

> “Chemotherapy makes doing the ordinary, extraordinary. The other side is that the ordinary is extraordinary. There is an appreciation for the everyday events of life. Taking a shower or eating dinner with the family are recognized as pleasures in life. There is satisfaction in doing daily chores. One even notices trees and buildings. They even seem beautiful.”

> “I was once asked by a physician, “What's it like (to be a patient with cancer)?” A strange question, I thought, coming from an experienced oncologist. However, the question is telling. It shows the difficulty some oncologists have in empathizing with their patients. It also shows the desire some physicians have to understand illness as it relates to patients. Younger children may not understand what life and death mean. They go through a lot of pain without knowing why. They wonder what will happen to their family once they are gone. For older children it is wanting to do everything, thinking you will not do anything. There is a realization that you have not been to Disneyland. It is not wanting to feel helpless, but needing help. It is wanting to talk to others, but not having many opportunities to do so. It is telling yourself little white lies to keep yourself going.”

> “When I got down, I also tried to figure out what would make things better. Sometimes it was wanting a hug. It's terrible feeling sick all the time. As soon as you start to feel good again, you are zapped back into misery. Other times, it is being with wonderful people who care or having an appreciation of family and wanting to have loved ones around you, but when you want them. There were times when it was hard to be a patient. At these times I felt confused. I wanted to be limitless but realized my time is limited. Somehow, I always seemed to have the desire to live every remaining moment.”

> “When a friend asked me what kinds of things I worried about, I replied: “It is hard wondering about death. Would it be easier to die? Is it worth it going through therapy? At times, it is just wanting to make it to tomorrow and forgetting about all those hard questions. Goals, like making it to New Year's, became big achievements. With each goal, you want to prove you can do it and to succeed in all that you do. It's hard never being able to get rid of knowing that you have something that might be gnawing away at you.”

### *Educate the Family*

> “Here I am a school teacher, but I can't keep track of what went into my daughter. Toxicities, side effects, and prognoses dance around in a confused manner. It took three or four cycles to understand what medications were given and when. I still don't know whether bands are added to polys or monos and eos to polys for absolute neutrophil counts. Each x-ray scares me. Will it cause her to relapse? It just might be the trigger for more cancer. If she has already got it, maybe more radiation will make more cancer cells.”

As a treatment plan evolves, it is necessary to be sensitive to the educational and cultural background of the family and patient. The use of simple language, often with drawings or illustrations, is helpful in the explanation of medical terminology of treatment or disease. It is wrong to assume that a higher educational background guarantees a rational and intelligent understanding of the facts. Many people have pockets of ignorance and irrational fears about cancer and find details of the disease and complex treatment plans confusing. Avoid the use of technical explanations and be certain that your words are clear. Again, repetition helps to clarify the treatment program and also to identify and eliminate anxieties.

The patient will be under the direct responsibility of a physician for only a short period of time, perhaps for the induction therapy or at the time of a surgical procedure. Most of the time, the treatment depends on the family unit. Parents must learn how to observe their child—specifically (a) what to look for, (b) when to call the doctor, and (c) when to seek help. This becomes especially important if the child receives care in another medical center so that the family can adequately inform the treating physician.

The family members need to know what the treatment requires, how to manage the side effects, how long they will persist, and what can be done to minimize the discomfort. Although they may get information from other families in the clinic or hospital, it is not sufficient to assume that these sources are adequate. In reality, most families find out how to minimize discomfort by trial and error, despite the best efforts of anticipatory guidance. Still, a review of the possible problems before a therapy will also return some control to the family members as they come to understand what to anticipate.

### *Communication during Hospitalization*

> “I was so confused for months. I guess someone was looking after my child. Residents and interns ran in and out every morning. Some seemed more interested in the TV cartoons blaring in the background or the nurses' notes than my questions. My primary fellow always stopped by to talk. She tried to explain what was going

on, but sometimes she had to ask whether or not the dentists or skin doctors were in. More doctors saw my child than I have ever seen in my life."

Breakdowns in communication between staff not only lead to practical problems in patient management, but for the family, they are also symptomatic of a lack of supervision and responsibility. The family's fear of being a victim of a system that is not attentive to all details is almost as great as the sense of helplessness in not understanding why that particular family was struck by childhood cancer.

The identification of a primary team to whom the family can turn for questions or grievances will dispel the fear that an endless array of physicians will enter and exit without coordination of care. In an impersonal hospital environment, knowing that a particular physician is responsible for coordinating advice from consultants and making final decisions dampens anxieties. Furthermore, the realization by the family and patient that they are not expected to understand everything at once and that there will be ample opportunity for further discussion reassures them that there will be continuity of care.

***Interactions among Families***

"The best thing about the old oncology floor was what my wife called the bus stop, next to the elevator. It was the common meeting place where there was no good reason to be. You just sat there and talked with other people who were living the same nightmare as you."

"I often think I never would have made it myself, if I hadn't met the other families. They were my lifeline. I learned so much from them, and many are now my friends. When I really needed to know the inside scoop on a problem, I went to my friends from the bus stop."

"Then, there were those who grabbed everything and didn't pay attention to others. There were a couple of times I nearly slugged one particular mother. She refused to think of anyone else but herself. Although the things were petty, I was so wound up it was hard not to get real upset."

The sentiment that "many people feel just as you do in these circumstances" is a powerful denominator for comfort. Placing the family members in the context of others' similar crises offers a type of solace and at the same time encourages them to reach out to other families. These relationships forged in the clinics and the ward are as important as those with the medical staff. They are also an important source of education, providing early warning or information about upcoming events often previously discussed with the medical staff but not integrated into realistic expectations.

This close congregation of families and medical staff, however, raises two issues that require caution. The first involves patient confidentiality, and the second is the potential for conflict among patients and families. It is easy to discuss specific issues about a patient's treatment or prognosis when everyone on the ward seems to know everyone else. This must be avoided to ensure that the essential confidentiality between patient and all medical staff is maintained. What families discuss among themselves is their own business, but medical staff have to avoid being drawn into seemingly innocuous conversations about other patients that may potentially violate the patient's and family's trust. During hospitalizations, families are in close proximity to other families and can't help but observe what happens to others. Because of this, families may also need reassurance that what happened to the child down the hall is or is not likely to happen to their child.

Interactions among the families can also be a potential source of interpersonal problems. The microcosm of an oncology clinic or floor accentuates every relationship, particularly if it is not harmonious; such disagreements between families or patients are the concern of the medical staff as well as those involved. Arguments or problems between families poison the environment and, without directly legislating rules, the medical staff has to guide the families. There should be no exceptions to any established rules for the ward or clinic because such action is subject to extensive interpretation, including special preference as a diminution of interest in another patient's or family's plight.

***Listen to the Family***

"I was so mad at the doctor when he nonchalantly blew me off and said there was nothing wrong with my kid. He was 102.5 $^{o}$F at home, but in the clinic he didn't have a fever. Because of low white blood cells they put him on i.v. antibiotics. When his blood culture came back positive, I was angry and glad. I knew he was sick and now I had proof, but I felt horrible because my son had to be in the hospital for 2 weeks."

Nothing is more frustrating for parents than to recognize a problem in their child and have the physician dismiss it or treat it as something trivial. Family members commonly take pride in possessing sensitive observational skills. The repudiation of such abilities may be insulting to the family. Great care should be exercised in correcting a mistaken concept or observation. Because family members live with the child 24 hours a day, they may be so close as to not appreciate the context of a problem; what is subtle or obvious to the staff may not be similarly appreciated by the family. However incorrect the perception is, families should always be encouraged to observe. As with procedures, they should participate but not bear the full responsibility of care. They care for the child at home and, therefore, need to be informed and observant.

Sometimes families may form a hostile alliance against some aspect of the medical system. Usually there are indications of an impending problem, but not always. It is important to stay in touch with the family's concerns, especially when they are clearly stated, to intercept and correct misunderstandings.

In times of crisis, many parents seem to have a "sixth sense," intuitively knowing when a child is severely ill. They may describe the child as "different" or "acting funny." Listen to the urgency in a parent's voice, and do not dismiss it. At the minimum, directly address their concerns and either verify them or gently explain the discrepancy. In the physician–family relationship, the family member must know that his or her word is trusted.

"In the 6 months following the diagnosis of my son, I started on medication for hypertension and my husband gained 50 pounds. We underwent 10 weeks of marriage counseling. These were side effects of my son's illness."

"When a physician asks about the parent rather than the patient, it is like a pat on the back. It means to the parent, "Hey, we care about you, too.""

Often, parents will need medical or psychological care as a result of the stress of dealing with their child's illness. Because their attention is so focused on the ill child, they may not recognize their own condition. Direct recommendations for care or treatment for parents are difficult. Parents' tolerance of discomfort or inconvenience varies, and distinguishing between what is tolerable and unhealthy is highly individual. By pointing out the chronic lack of sleep or weight changes, the physician can inform the parent of the concern. Recommendations for the health of the parent may be misinterpreted as further control by the physicians over the crisis, and unless the medical circumstance requires immediate attention, specific action should be left to the discretion of the parent.

"Do you know what my husband talked about with the other fathers during a summer camp for cancer families? Insurance. The fathers spent the entire boat ride discussing insurance. They told stories of how frustrating and demeaning it was to have to keep worrying about money when their child was fighting a life-threatening illness."

Once a child is in remission, there may be a tendency to downplay the nonmedical problems and be less sympathetic to problems of insurance or school-related issues. Unfortunately, for the patient and family, these problems are a constant source of frustration and considerable anxiety. Many parents face the uncertainty of job loss because of extended absences to care for the family. They may be treated differently and lose professional opportunities for advancement because they are "not able to fully devote themselves to their work." Offering a sympathetic ear to these problems reaffirms the staff's commitment to the patient and their family. In addition, a short note or telephone call may help to preserve a family member's position in the community or workplace.

***Social Awareness***

"Soon after our son's diagnosis of a medulloblastoma there was concern on a skeletal survey that he may have had involvement in his hip bone. A CT scan was ordered. We were understandably very upset as we set off for the radiology suites, and as we left the clinic, we passed one of the staff members. He had seen our son on occasional rounds and was fully aware of his condition. He greeted us, inquired where we were going, and when we told him, "To a CT scan" and why, he replied cheerily, "Have fun." As well-meaning and innocuous as this remark may have seemed, in this circumstance it was inappropriate. No one has fun having a CT scan, especially for the above reason."

One must always be sensitive to the plight of the family. The above vignette illustrates how a seemingly innocuous remark in the wrong context can be disturbing to

the family. In some circumstances, polite small talk may be deleterious when the patient or family perceives that the situation is being trivialized.

Still, small talk and humor, when appropriate, are effective in deepening the trust in the physician. Naturally over the extended course of treatment there will be times when everyone relaxes and jokes and games are a welcomed diversion. The common enjoyment of a laugh or anecdote breaks down the patient–physician barrier. During that moment of relaxation, a child may see the physician as more than a symbol of the illness. The awareness of another, more human side instills trust.

Children often interact with medical staff in the role in which they see them. A nurse is just a nurse, with no other life. A doctor could not be a spouse or parent. They are perceived in this limited role and expected to be attentive to the child's needs. When a child can identify the physician as a person, perhaps with a family or an outside interest, he or she may think “Oh, you are going home to someone like me or my parent,” and thus be more accepting of the physician's absence. This is of particular importance when the patient and family are more demanding.

#### *Telephone Contact*

> “When the doctor called to tell me that we needed to perform an extra x-ray at our next appointment, my heart sank. I found myself sweating and nervous as we talked, wondering if there was something she was not willing to tell me on the phone. I was too afraid to ask why it needed to be done. When we arrived at the appointment, we were met by our favorite nurse, who immediately put my anxiety at rest when she indicated the test was to be done to follow-up something that had resolved. We did the test, it was fine and we went on. Still, every time we get a call from the medical center, my heart stops.”

As helpful as the telephone may be, it is also intimidating. Without the ability to “read” the nonverbal cues of the physician, it is harder to interpret what is said. When calling a family at home, remember that a common first reaction is, “Oh my God, what have they learned?” The family has already been surprised before. If the information is perfunctory, quickly acknowledge so. Do not leave the family guessing as to whether there is a hidden agenda to the call. Conversely, if there is a need to convey alarming or distressing news, be certain that you are in a quiet place with the opportunity to talk freely and extensively. It may also be prudent to cue the parent by asking, Have you got a few minutes to talk with me? This also permits the person to arrange for the elimination of distractions. At the end, offer the opportunity of a meeting.

#### *Internet Resources*

> “I told my doctor that there was a new cancer therapy shown to be effective in mice. I heard about it on CNN and then found a Web site describing the study. It cured my child's type of cancer overnight in all the mice tested. When I asked if we could be the first family to receive this, she kindly put her hand on my shoulder and said, “Let's take a deep breath and find out more. There are big differences between mice and people. Furthermore, there are differences between people.” She offered to sit down and explain her understanding of the current study after she had a chance to read the article and talk with her colleagues. By the time we sat down a day later, I was keyed up. She explained the complexities of the research and suggested that we not do my son any harm. While I had fantasized this was the magic bullet, it dawned upon me that no person had ever received the therapy. Was I going to let my child be first? Only if it was for certain. Later, I was thankful for her quiet and supportive comment not to go too quickly.”

One of the newest challenges to the health care team is the availability of unfiltered information on the Internet. Nearly every family, either directly or with the assistance of others, surfs the Internet in search of information. The vast majority of the information is exceptionally helpful, providing resources for access to care and support groups, primary information on current therapies, and background on the staff. There is no question that families are better informed. This does not mean that they are better educated, however. Extremes of opinion and individual testimonials create attractive alternatives to offered medical plans. Even more patience is required when confronted with data on alternative or untested treatment plans. Sometimes it takes great resolve to receive the information and offer to respond at a later time. We cannot forget to communicate to patients and families that information must be critically evaluated and not naively accepted on face value. One of the most difficult tasks is to navigate the way between maintaining an open mind and providing guidance that will keep the patient on track for appropriate therapy. Again, keeping open the lines of communication with regular meetings and discussions should help to integrate information gathered with the best of intentions.

It is expected that the quantity of available of information on the Internet pertaining to childhood cancer will continue to grow exponentially. Families will undoubtedly use its resources throughout treatment and survivorship, and for this reason, care providers can expect to address questions arising from a wide spectrum of sources—some credible and others suspect. Perhaps early in the establishment of a relationship with the family and patient, it might be prudent to offer concrete guidelines on how to approach Internet information. This could be one of the major topics of discussion of a family meeting once treatment has started.

Some useful recommendations include the following. First, encourage the family to examine the authorship and sponsorship of the Internet site. Close attention should be paid to the clinical and scientific advisors of the Web site. It is important to identify an internationally recognized group of consultants who provide expertise and guidance listed directly on the site. For example, a recently developed Web site ( http://www.cancersource.com/) provides current information on-line; here one finds an advisory board, which provides oversight of posted information and links. An additional sound practice is to use sites officially maintained by internationally recognized organizations, such as the American Cancer Society or the National Cancer Institute. The assimilation of information disseminated by national organizations should ensure provision of timely sources and the comfort of useful information. In a similar manner, families can also be referred to national advocacy groups, focused on specific pediatric cancers, that seek to provide pertinent information and network families facing comparable, but not always identical, challenges. When possible, it should be emphasized that there is a difference between supportive programs, addressing the needs and life of the family and patients, and treatment programs. The latter is best discussed and reviewed with the knowledgeable medical staff, whereas in the former the medical staff is only peripherally involved, insofar as it pertains to delivery of medical care.

> “I ran into the doctor's office to tell him that we had to begin a new hormonal treatment in conjunction with my son's anticancer treatment. I had found that it cured 9 of 11 patients according to a Web site and the treatment plan was now available for everyone outside of the United States.”

> “After I spoke with the doctors, who then looked at the site, it was clear that this “natural hormone” was an over-the-counter compound with no proven track record. The fine print indicated that it had been given to patients with a type of skin cancer that is treated by surgery alone, making the data even more suspect.”

In most cases, sites that promote a treatment or program require close inspection, specifically looking at whether a product or treatment is being sold on its commercial merits and not necessarily on its scientific or clinical track record. One of the most daunting challenges is to answer questions based on sites offering testimonials on new or alternative treatments. Often, it is necessary to take the time to explain the difference between an established therapy and anecdotal experience. In doing so, it is critical to be sensitive to the family's yearning for certainty, something that cannot be guaranteed, contrary to what is often implied in or inferred from a Web site. Herein lies the dilemma, effecting a balance between encouraging an active participation in all facets of the care and critical evaluation of information, whether it appears to be sound or unproved.

## MAJOR EVENTS IN THE CARE OF PATIENTS AND THEIR FAMILIES

### Shock at the Time of Diagnosis

#### *The Patient*

> “I remember being in an examination room by myself because the doctor walked out with my parents. I could hear what they were saying but all I could understand was the doctor, who said, “Your child has only a few months to live.” My parents returned to my room, very distraught. The doctor tried to be kind to them and to me, but he forgot how thin the walls were. I knew I had cancer, but there was no way I was going to die. The surgery should have cured me. The next thing I knew I was off to another center where everything seemed big, including the desk and the chair. I felt younger than my age. I left the room while my parents were inside, and when they came out, they told me the doctor was more helpful.”

No one should hear that they have cancer or other bad news inadvertently, especially a child or teenager. In addition, the patient should not be alone; family members provide immeasurable security. Sometimes, the diagnosis comes as a complete surprise, whereas in other circumstances, especially with older children and adolescents, it may be the confirmation of a long-standing concern. Including the child in the initial discussion highlights the significance of the problem, and in doing so, children may sense the gravity of the moment when they see an upset parent or sibling. Although a patient is scared and afraid, in the comfort of the family, they may feel an important sense of security and protection, especially from the threat of further pain or discomfort. In this circumstance, the child may also sense a loss of control imposed by therapy and ts toxicity and yet not understand the specifics. The challenge of the medical team is to instill a sense of purpose for all interventions.

It is remarkable how quickly children mature in the face of adversity. Despite their illness, they are often the strongest member of the family, with their sense of purpose carrying the distraught family.

> “While I once was in getting chemotherapy, I saw a physician interview a 6-year-old girl in a sweet, melodious tone. After about 3 minutes, the girl stood up, put her hands on her hips, and said, “Don't talk to me like I'm a baby.” I also met a 6-year-old child whose favorite thing about a fingerstick was that the hematologist made animals out of the bandages. Some doctors get fooled by the age of a child. Cancer makes some of us grow up fast.”

> “I was astonished at how cooperative my son was with his treatment. He'd always been stubborn and self-willed. It cost him a lot to give up so much control, but I think he did it because he knew this was really serious.”

In most circumstances, shortly after the diagnosis, the physician should talk with the patient in a calm, nonjudgmental manner befitting the maturity of the child. If there is going to be further therapy, explain why—that is, that there is hope that the treatment may cure the cancer. The prognosis should be given honestly but with as much sense of hope as possible. Be careful not to let the tone of the presentation, however, whether it be encouraging or discouraging, overwhelm the message. For example, excessive hope can misdirect the family and patient toward unrealistic goals. Hope is always needed, but it must be tempered with a realistic portrayal of what can be expected and what is unpredictable. Still, patients may believe that they will overcome their disease, having no question in their mind that they will be the survivor. It's clear that having room to hope creates better outcomes for patients in every situation.

Judging how to speak to a child requires great sensitivity to the child's maturity and to the cultural and philosophical background of the family. In many circumstances, families may request that information be withheld or delayed for a variety of reasons. In this setting, exploration of these motives may shed light on the families' biases and lead to an improved understanding between family and physician. It may also highlight sensitive cultural issues of which the physician needs to be aware throughout treatment and afterwards.

***The Family***

> “I will always remember 4 days in my life: the birth of each of my children, the day President Kennedy was shot, and the day my younger son was diagnosed with leukemia. That day will always be frozen in my mind. I remember the color of the tie of the doctor, the nurse's shoes, and the box of Scott tissues. Everything stopped. We talked to the doctor and nurse for what seemed like 15 minutes but was actually 2 hours.”

> “I would suspect that many other parents, like us, show classic symptoms of shock at the news of a cancer diagnosis. I remember spending a lot of my time in our first review of the protocol willing myself not to cry; my husband lost his voice. I've heard other parents say they banged on the walls and became hysterical.”

The announcement of either a presumed diagnosis or a confirmed diagnosis of cancer is imprinted in the parents' memory for the rest of their lives. From the time that the word *cancer* is understood, shock immediately sets in. They may hear very little after that point while their worst fantasies race through their mind. Is my child going to live? Will it be painful? Is it my fault? Did I do something wrong? Why is my family affected? Such questions naturally flash through the parent's mind as the discussion of the diagnosis continues. It is important to acknowledge each question as legitimate before answering. Many times, a question is not asked directly. Paraphrasing the question before answering it also helps recognize the importance of a query and provide an answer that the family may be concerned about soliciting. Some parents worry whether it is appropriate to ask these types of questions at this juncture, and it may be helpful to introduce such questions or point out that many parents “wonder what they did wrong,” or ask, “How could this have happened to me?” There may be a need to assuage feelings of guilt with a reassurance that “it is not your fault that your child has cancer.”

Postponement of these questions is a delicate matter because they are of great immediate concern. The survival of a child is now threatened, and the parents and patient want to know the answers. There is a strong desire for the answers to somehow be better than the anticipated worst-case scenario envisioned by the parent and patient. At this time, there is a natural dialectic between wishing this news were a bad dream and the instinct to immediately do what is necessary to correct the situation. In addition, this complex set of emotions clouds the parent's ability to think clearly and rationally.

The first conversation is critical because it sets the precedent for all subsequent conversations. At a later time, each word will be carefully analyzed and each movement interpreted. For example, “What did he mean when he looked right into my eyes and said, 'We'll win this battle'?” The physician has to be careful not to force the conversation beyond what can be understood by the family and patient at that moment. In this regard, it might be helpful not to get bogged down in what-if questions. Answer directly to provide reassurance, but at the same time, steer the conversation away from dwelling on every possible deleterious outcome, keeping in mind—and reminding them, also—that there will be ample opportunities to address each eventuality as needed.

Stressed parents need to feel that all of their concerns will be addressed. Even though their lives have changed drastically in a brief amount of time, they immediately need to identify someone who will educate them while treating their child. Their sense of loss and disorientation is frightening. The knowledge they must quickly acquire is a double-edged sword. It is important to know what will happen, but it is impossible to envision the nature of the anxiety and pain that they are about to experience. Later, parents remark that the fear of the unknown is the most unsettling aspect of the first months. In the midst of this time of crisis, turning to someone in whom they must place their full trust puts them in a vulnerable position. They hardly know the physician, yet they are now entrusting this stranger with that which is most precious: their child.

During the initial shock, many parents may need to “be taken by the hand” and concretely directed. It is virtually impossible to plan ahead when paralyzed by the initial diagnosis. Introducing the entire oncology team with its full complement of support staff is especially beneficial in the first days. It demonstrates a commitment to the total care of the child. In the atmosphere of crisis, concrete direction and assistance help the family to organize their priorities. Knowing the specifics of the social worker's schedules, parent support group meetings, play therapy activities, and sibling support programs send a strong signal; these are essential components of the comprehensive care of the child. For the quality of the family life, these may be as important as any other support mechanisms.

***Siblings***

> “The second hardest thing I ever did was tell my son that his sister was sick and might die. He looked at me and said, “No, she won't. I'll help her.” ”

One of the most difficult conundrums for the physician is when and how to address siblings. Families have their own style of discussing important matters, which must be respected. Infringing on family members' ability to talk among themselves may be interpreted as another example of a family's losing control over its own affairs. Hearing the diagnosis and treatment plan from a family member or friend may permit the sibling to react without embarrassment and to ask questions freely.

Once the family has told the siblings, they may want the doctor to speak directly with the siblings, either alone or in a family meeting. It may be an appropriate opportunity to raise the issues of turmoil in the family structure, as discussed above. Opportunities may also arise to directly counsel the sibling that the child with cancer may receive most of the attention, at their expense, but that it is no reflection of lost love or affection.

> “I talked to a friend who was the sister of someone I knew on the wards with leukemia. Even after 4 years, her eyes teared up when we talked about her brother's relapse treatment. It was hard for her to think of her brother's suffering. She always felt helpless and while she tried to help, she was also an outsider in the care of her brother.”

Siblings may feel alienated and distraught. Often, they are scared and may even assume a feeling of guilt and loss. They need to be taught how to deal with both the emotional and practical problems of a treatment plan. At some point, an understanding of a protocol and its implications may help focus the child on assisting in the care and may explain why there may be long waits or frequent visits to the hospital or ward. Throughout care, siblings require an affirmation of feelings and a dispersal of guilt. These issues are also applicable to the extended family and friends. Early in the course of therapy, it may be helpful to inquire as to whether there are other significant friends or family members who may be called on to help administer care or provide support for the patient and family.

**Presentation of Treatment Plan**

> “Our first conversation with the doctor and nurse overwhelmed us. Here we had just learned that our son had a tumor and the next thing we knew we had a complete protocol detailing the next year of our life thrown in front of us. When we finished talking, we went downstairs for coffee and spent the next hour going

over every word, looking for hidden things. For weeks, we talked about the contents of the meeting and the protocol."

"Everything came at us so quickly. We had to sign a protocol the first day and start therapy that night. It seems like they took away all control of our lives. We would be ruled by the protocol for months. What bugged me was that someone else wrote this thing that our doctor was going to follow and we had to follow what he told us. It took a while before we had any idea of what was going on."

The presentation of a protocol or therapeutic program is a threatening event. The patient and family have had little to do with its development and want less to do with its implementation when they learn of the side effects and toxicities. Furthermore, when the support data, intended to convince the family, are reviewed, there is great concern that their child is "just another statistic" and not a unique individual. Sometimes it bears repeating that a protocol is not "written in stone," and treatment is administered to the individual as needed. It requires great effort to clarify that the protocol or program is intended for the individual child and will be modified as problems arise. Lengthy discussions of data may confuse the parents' ability to understand what is specifically going to happen to their child. Families are rarely capable of discerning the relative importance of information.

This problem is even more acute with patients, especially when they are adolescents who are mature enough to participate in the discussion but do not fully understand many of its implications. Although many family members desire to know the statistics, these numbers must be carefully explained and put into an appropriate context. If the patient and family do not believe that the treatment plan is designed for them, it may undermine both their compliance and their relationship with it. It is important for patient care, however, as well as for obtaining informed consent, that the family and patient be well informed about the proposed therapy. Specifically, it is helpful to explain the findings from previous studies and, in doing so, to define the terms *cure*, *response*, and *relapse*. These definitions may, however, seem arbitrary to patients and their families and in conflict with the quintessential question, "Will I be cured?"

The key to the presentation of a treatment plan is a sense of hope. This may be the hope of "cure" for some or "palliation" for others. Regardless, it is important that the treatment plan be grounded in hope. If the treatment plan holds little or no hope, then it is in the best interest of all parties to explore alternatives.

In the initial discussions, it is also important to convey the practical ground rules for administering and monitoring therapy. Often, these issues may be too voluminous for the families to assimilate in the initial encounters. It is too much to expect families to grasp the subtleties of drug toxicities or the importance of peripheral blood counts in determining the next therapy. As abstract as these details may be in the initial discussion, however, they quickly become practical issues readily comprehended by the family once therapy begins. Still, during the initial discussions, the medical team must be sensitive to the difficulties that families have in listening to that which is often routine for the medical staff.

**First Therapy**

"When I entered the room for my first treatment, I met a veteran of the ordeal. He was upbeat and kindly told me what was going to happen. He said that I'd smell something funny; that would pass; later, I'd feel nauseous and throw up. I was happy to meet my roommate, but I did not realize the implications of what he told me. I could barely remember ever having thrown up before. The doctor pushed the medicine, which gave me a strange sensation in my nose, and then I felt fine and confident for about 10 minutes when I decided to go to the bathroom. No sweat, I thought. Suddenly, as I closed the door, the whole of my insides exploded. I threw up all over the floor. As I got back in bed, I lost control of my bowels. For the first time in 14 years, my mother had to clean me. After another 5 minutes of it, even that did not disturb me. All that I could manage was to simply exist. After 2 days of being forced to drink and urinate, I developed mouth sores, which left me only able to drink through a straw."

The side effects of a drug may develop without warning, catching patients and parents off guard. During the violent onslaught of emesis, pain, and diarrhea, they may also be confused in their thoughts. In spite of being forewarned, the depth of experience is not appreciated until the first therapy or later. The above story highlights a particularly troublesome problem—loss of bowel control. This may shame the younger patient who has recently mastered it and embarrass the older patient. It may result in a loss of self-confidence and self-worth.

"Another thing that doctors can do to help during the first treatment is simply hold the patient's hand. It makes you feel comfortable to have someone touch you. Although you usually want your parent, you may not want to be embarrassed in front of them. After all, the doctor gave you the medicine. He or she should be there to see what it does to you."

The presence of the physician during the first therapy and its immediate aftermath is reassuring to the patient and family. It is a strong statement of compassion. Physicians, like anyone else, dislike watching people throw up, but this is the time for the best "poker face" possible. The child does not want to be perceived as disgusting. It would be better if he or she saw that physician who administered the toxic therapy was present, giving a sense of reassurance and support. Children are remarkably apt at discerning whether the physician is genuinely concerned. The first therapy, like the discussion at the time of diagnosis, sets the precedent for all subsequent therapies. At the next visit, when the patient has recovered from the side effects, it may be prudent to discuss the specifics of the first therapy with an eye toward developing the optimal conditions for future treatment.

**Keeping Track of Treatment**

"We always came to the clinic visits with the latest volume of the daily diary. Every day's dietary intake, wake up time, temperature, bathroom visit, and medication were meticulously tracked. When our son was admitted with *Pneumocystis carinii* pneumonia, we were able to look back and see the subtle changes that were taking place over the week before. We also had written in the margins of our diary that he was more tired than usual and had a hard time at school."

"Our medication calendar (we crossed off each medication after it was given) was the only way we could make sure our child followed his protocol. It was just too complicated to keep track of any other way. We also noted side effects, so we could pinpoint when to expect problems."

Treatment plans are often difficult to follow, regardless of the educational background of the family. Families appreciate gentle reminders but may resent directives or orders. Encouraging the patient and family to assume responsibility for the administration of therapy may also counter the loss of empowerment, and by focusing on a pattern, patients and families are better able to reestablish a daily routine.

At the conclusion of a visit in the clinic or hospital, a parent or patient will express frustration that he or she cannot remember questions or problems incurred at home. Recommend that any comments or questions be written down, perhaps in a diary or notebook. This strategy is especially useful for routine items, such as when sun exposure needs to be avoided with some therapies. Furthermore, medical diaries with laboratory values and subjective comments may provide invaluable assistance in the medical management of the patient. The detailed information wrests some of the control back from the physician, because the parent becomes the keeper of important details, some of which may be cross-referenced with a chart or protocol.

**Procedures**

"Whenever I have to have a bone marrow [biopsy], I feel like a machine. "Turn over and let's get started." For me, it hurts no matter what you do. I like having my mom there with me. She helps me before, but when it starts, she can't stop it from hurting."

Because the parents know the child best, they may have helpful input for procedures. For example, the staff member may encourage the parent or older sibling to "help" with a procedure. This may take the form of holding a hand or bottle of intravenous fluids. Great care should be taken not to pressure the parent or sibling. In other words, they should be encouraged to the degree that they are comfortable, but at no time should they be perceived as active participants in the procedure, particularly if it may be painful. In some cases, the parent or sibling may need to leave the room so that they may be received as comforters, even if it implies that the staff are villains. It is better for the staff to assume this role, because they do not go home with the patient. Every effort should be made to discourage the perception that the parents are responsible for the pain and discomfort.

After the first procedure, it may be worthwhile to discuss its specifics—how the procedure was prepared and performed with an eye toward modifications that may ease either the pain or the anxiety, both on the part of the family and the patient. Allowing the patient and family to develop a routine or ritual returns some of the control to them. It is also a statement of individual will. In a sense, the patient and family have wrested it back and it should be protected as long as it does not pose a risk or make an impractical demand.

> “On about the fourth or fifth spinal tap, the doctor couldn't get in. He tried three times and during each attempt, my daughter wiggled and cried. He tried but couldn't do it. He had done it each time before without much discomfort. In fact, we used to come to clinic fearful that someone else would do it. But when he couldn't get it, he said so and left the room to find someone else. During that time, I wondered if he'd ever do it again. Would anyone else be able to do it? ”
>
> “He reentered with one of the more senior oncologists, who had trouble, but got in on the second try. Afterward, our doctor apologized and explained to my daughter that sometimes spinal taps are hard to do. He asked that he be given another chance at a later time. I must say I was conflicted. The next time round, I didn't know whether I would let him, but when the time came he talked us through and it worked on the second try.”

Competency in performing medical procedures is another source of great concern. The last person to do a lumbar puncture or bone marrow aspiration with minimal discomfort is frequently invoked as the preferred choice. The inability to perform when previously successful requires humility on the part of the physician. Seeking another physician before it becomes an impassioned plea may be difficult to accept, but for the long-term relationship, it signals a standard of honesty and humility appreciated by the family. Admission of the failure along with a promise to find another physician may also diffuse a potentially volatile situation in which the physician's trust may be undermined. Just as with each conversation, each procedure assumes great significance in both its technical performance and its expected result.

> “Why can't I have the bone marrow test instead of my son? ”
>
> “It's very difficult to watch your child experience “planned pain” and be unable to stop it. You feel so helpless and inadequate.”

During the discussion and performance of medical procedures, the recognition of the loss of control will make the physician more sensitive to the parents' predicament. Parents occasionally express deep guilt and state, “Why can't I be the one to undergo a bone marrow aspiration?” They, too, need a routine so that they may be prepared for enduring the procedure as well as the wait for the results.

The preparation for a procedure, such as a bone marrow aspiration or a computed tomography scan, is a highly volatile time because the family is both fearful of a discouraging result and of the response by the child. How a family prepares is highly individual. Unless a family routine is disruptive to a clinic or floor, every effort should be made to accommodate the patient's and family's schedule requests. Special circumstances, such as relatives' birthdays or school or social events, may dictate alterations in a schedule. To the patient and family, the accomplishment of participating in one of these events is a triumph. Its achievement may seem as great as the endurance of therapy. These milestones serve as goals to be attained, and when they are, they represent a kind of victory over the influence of cancer on their lives.

**Waiting**

> “As a general rule, patients are willing to wait about 15 minutes; after that, they get annoyed. At 30 minutes, they get upset. Approaching an hour, they are livid. Any longer and they become hostile.”
>
> “Mom always gets upset when we have to wait. I tell her to stop doing it because I don't like it.”

Children have a tendency to be more tolerant of waiting than their parents. Parents grow more anxious with time, because sitting in a waiting room is both frustrating and frightening. Waiting brings out their helplessness because they must be available when the doctor is ready. Although it may serve as a reminder of their loss of control, it may also be interpreted as indifference or a lack of respect on the part of the staff. Some parents may even fear that it is a judgment on their child or a minimizing of importance of the child's care.

In the setting of the clinic or hospital, acknowledgment and an apology are required for lateness. One should also be prepared for an element of hostility, because the frustration may contribute to passive-aggressive behavior. The parent's level of anxiety may increase during the wait, and extra time or explanations may be required for the visit to be satisfactorily concluded.

For the child, on the other hand, a playroom full of friends and activities may offer a retreat from the dreaded visit to the doctor. Children do not possess the same sense of time as adults, partly because they are more concrete and live for the moment. Worse for both the parent and child is when the child needs to be admitted to the hospital for a particular test. Even though the purpose may be “routine,” the child is in the hospital because there is enough of a risk of complication to require observation in hospital.

> “Time is more than a commodity in a hospital; it is a call to think worrisome thoughts. Any excuse to leave a waiting area is welcomed; it frees you from having to sit and worry.”

If a delay is anticipated, call or leave word so that the family members may move about, whether it be to get a cookie from the cafeteria or make a telephone call. If, by chance or bad luck, the parent is off the floor during a daily hospital visit, try to return or at least speak on the telephone with the parent later that day. The daily contact is reassuring and conducive to further questions.

> “Every time we came to clinic for a routine bone marrow aspiration, it was like having lunch with an atom bomb. Everything was supposed to be okay, but everything was supposed to be fine when my daughter went to the doctor for a cold and ended up with leukemia. Waiting around is so hard. I used to get so worked up until they told me the results.”

It may be a routine blood cell count or bone marrow aspiration for the physician; but for the patient and parent, nothing is routine. In particular, the parent may have been concerned about the test for the preceding month. Each test has the potential of bringing further bad news. Patients and parents resent the use of the word *routine*. The family has already been shocked at the time of diagnosis, and the possibility of more unpleasant “surprises” lingers throughout therapy. There should be a clear indication for each test that should be understood by the patient and family.

If there is an unanticipated delay in reporting the results of a test, inform the patient and family of the delay as soon as possible. Unexpected delays are usually equated with bad news; and the longer the wait, the greater the apprehension. Reassurance that a particular delay is not related to an unfavorable result will greatly help parents through test days. When a result is available, it should be delivered in an environment where discussion may ensue freely. Even a normal result may lead to a series of questions, so be prepared to discuss more than the individual test result.

**Back to School**

> “It seems like everybody I saw back at school knew my whole medical story, even before I said two words to them. Some were up front and asked me questions, while others seemed to look at me funny if I coughed or sneezed! Having to explain my diagnosis, treatment, and the side effects, like my thin hair, got to be tiring very quickly. Thank God my good friends got used to it and it didn't seem to faze them. Others were so uncomfortable that I would touch them and say “Look, I won't bite!” ”

The stigma of a cancer diagnosis is a formidable challenge to the child or adolescent returning to school, who may be bald or look very thin. Encouraging the return to school with specific advice and support initiates a positive step toward the reconstruction of a shattered life. Particularly for children, the reestablishment of friendships and social acceptance is gained through common experiences that can only take place in the company of other children. Advice to “participate as tolerated” should be tempered with the caveat that a gradual resumption of normal activities may take time for a variety of reasons. It is hard for a child to hear that they are not ready to do something, especially when they see friends active. Instead, it may be best to offer a challenge and provide conditions that may encourage eventual success. One of the hardest things for a child with cancer to encounter is outright failure in resuming activities. The couching of future plans in terms of providing guidance and pacing will help to rebuild self-esteem and gain the confidence and respect of friends and family.

The decision to resume school-related activities is a major sign for families to gain a sense of control over the diagnosis of cancer. Help families to seek the proper supports in and out of school. In particular, the social worker and school guidance counselor can facilitate academic and social reentry. Especially at the time of

reentry, families are thankful for an extra word or intervention from members of the health care team. It relieves them of the necessity of explaining a painful situation.

**Concluding Therapy**

> “When my son finished ALL [acute lymphoblastic leukemia] therapy, we had a pool party. All of our relatives and friends came to celebrate the end of a 2-year nightmare. We invited our oncologist and his family to come, but he declined the invitation.”
>
> “He said “that it was not appropriate for him to come. There was still plenty to be done. Besides, we still had to do more bone marrows.” ”
>
> “At first, we were hurt. If it were not for his doing, we wouldn't be having the party, but then we realized that we weren't out of the clear. We still had the party and the off-therapy bone marrow test was fine!”
>
> “Every week after therapy finished we called the clinic over some matter. Until we had the first CT scan off-therapy, we lived on pins and needles. I slept worse than during radiation therapy. I couldn't believe that we were finished. It was the wait that was so scary. Everyone in the family had nightmares, and in the morning we all seemed so grumpy.”

There is great anxiety at the conclusion of therapy. During therapy, some parents describe a “certain security” with the administration of therapy. Something is being done to combat the cancer. When therapy ends, the patient and family have to wait for a period of time before evaluation confirms remission or relapse. Remember that success is measured as the absence of failure. It is during this wait that there may be great anxiety and frustration. Although there is no way to diffuse the anxious anticipation before the upcoming studies, the attention needs to be directed to the reestablishment of a routine independent of clinic visits or a medication schedule. The family unit has to relinquish dependency on the medical staff and begin to regain the control lost to the demands of the therapeutic program.

As therapy concludes, it becomes necessary to educate the family about sequelae of treatment. Although long-term effects may have been covered around the time of diagnosis or initiation of a new protocol, this information is then perceived in a different way. Surviving therapy is not easy. It has been the goal since diagnosis, but now the patient and family face the future of its consequences, most of which were not fully appreciated at the time of diagnosis. Many families will remark that if they had understood the nature of the side effects beforehand, they may not have consented to therapy. Just when they thought the nightmare was over, they have to confront new concerns, some of which may be permanent. Similar to the series of questions that run through the mind of a patient or parent during the discussion of the diagnosis, these should be explicitly addressed. It is important for the family to realize that the care of the child has not concluded with the last therapy. There are concerns that will be active for years to come. Early detection and management of long-term effects may be improved by a well-informed family.

> “Well after therapy there are some things that remind me of the experience—for example, the smell of rubbing alcohol. I knew of another patient who got sick to his stomach every time he saw a particular red color that reminded him of Adriamycin.”
>
> “When I finished therapy I was happy I had had cancer and got to meet many great people, but with time I started to think surgery, radiation, and chemotherapy were just awful. Even now there are memories and odors that tense my stomach up. Surviving takes time, but you do regain your old body, your hair grows back, and you go on with life. There are times when I just don't want to think about it or even think that it ever happened. All I have to do is look at the scar on my stomach and it hits home.”

**Relapse**

> “When the doctor told me that I relapsed, I already knew it. It was almost as if I was hearing it for the second time. Something wasn't right. I kept feeling tired when they told me I should be feeling fine. I couldn't do what I was supposed to do. I knew it was back, but I didn't want to find out because it would ruin everything all over again. I was back at school and my hair had grown back. Everyone at school had forgotten what happened, and I was having a great time.”
>
> “The worst thing that a doctor said to me when I relapsed was that I wasn't going to be able to do certain things. I was so mad at him for saying that. I still went ahead and did things that he predicted I couldn't, like go camping and swimming after my amputation but during my chemotherapy!”

Relapse rarely comes as a surprise to the family unit. Since the day of diagnosis, when no guarantees were issued, the family has lived under the specter of possible recurrence. When there is suspicion of relapse, be explicit in stating your concern. More often than not, the patient perceives something is wrong. A child should not be surprised, nor should he or she be the last to find out. Some parents express the need to know whether to quickly prepare for the recurrence of the whole nightmare. Nothing hurts more than the surprise of relapse, particularly when there is enough concern that they may look back and wonder why they did not know earlier.

When relapse is diagnosed, the patient and family grieve over the failure of therapy. There is little that is hopeful in relapse. Even though the odds of survival at the time of initial diagnosis were known, to sustain themselves patients and parents have believed that they were going to be part of the “good” percentage, no matter how high or low. Nevertheless, throughout therapy, there was always a gnawing concern that therapy may fail. Relapse confirms this worst fear. The hope that sustained them throughout previous therapy has been tarnished and, in many circumstances, destroyed.

> “When my child suffered a relapse of her cancer, I was shocked to learn that some of the same drugs would be used to induce remission. My first reaction was to disbelief. How in the world could they use these drugs again? Did they not use them correctly or did they come from a bad batch? Was it the medical staff's fault? These questions kept racing around. When I finally calmed down, we listened, were convinced of the importance of the offered therapy, and now, three years out, we are free of cancer.”

The physician is handicapped because previous therapy has failed and the medical staff may even be blamed for the failure. Whether this is articulated or even consciously believed, the physician should be aware of this sentiment and perhaps address it directly, particularly with younger children. Adolescents and family members may become accusatory and bitter toward the medical staff. Conversely, many families state that they looked for pity and that they sought solace from those who understood: the medical staff and other families. Some families may even expect the physician to be more involved, not only to oversee the details of therapy but also to support the family, almost as a gesture of atonement for failure of previous therapy. Others may be so angry as to exclude the medical staff whenever possible.

> “After my son relapsed, one of the most insensitive and upsetting things said to me was, “Oh, you can fill in your signature on this consent form. You've been through all of this before; it must be much easier the second time around. You know the side effects and what to look for.” ”

This type of comment is very disturbing to the patient and family constellation, who are scared and upset. Comments about being a veteran, or “knowing what it's all about,” are disingenuous and hurtful. Furthermore, the sense of failure is heightened, and some patients fear being closer to death.

Although patients and their families have lived through one therapy, it is important to point out the differences among therapies. Families may be veterans of a protocol, but this does not imply that they will understand a new protocol. They are frightened and upset by the relapse and may not grasp the subtleties or the overall plan any more efficiently than at the time of initial diagnosis. In many circumstances, even more patience and empathy are required in explaining a treatment plan. Previous therapy will undoubtedly bias them and in many circumstances work against a realistic understanding of treatment of the relapse.

The family members must prepare for reliving the pain and suffering of treatment, whether it is curative or palliative in intent. Once again, the family has lost control and must endure everything over, but this time it is a recurrent nightmare—one that will not go away and is about to become worse. Each event of the calendar is more painful. Before relapse, there was a sense of accomplishment in passing each point of treatment or study. Now, it is back to the beginning, but the situation is further complicated by several elements: poorer likelihood of survival, further toxicity, and long-term complications, as well as the dreaded knowledge of some events to come.

> “Well after the time of relapse, it really hit home when the doctor offered a trip to Disney World. That's when I knew things weren't going well. All I could do was hold back my tears and say, “We'll do whatever it takes to make my son happy.” ”

One of the most difficult dilemmas for the physician is the transformation from curative to palliative therapy. It is a brutal change of attitude for the family. To them, it is

a painful reminder of the expected loss of the child. How and when this concept is understood by the family and patient is highly individual and, naturally, requires insight into the dynamics of the family. Nonetheless, the physician bears the ultimate responsibility for clarifying the intent of the treatment—curative versus palliative.

At a significant juncture such as relapse or death, a physician can never afford to forget that the interaction with the patient and family represents a small portion of the child's life experience. Although the consequences of a pediatric oncologist's care may be great, each child possesses a rich wealth of experience that is shaped by the family customs and his or her cultural, social, and philosophical milieu. Particularly at a time of crisis, other important influences may need to be consulted and may include a wide range of individuals: clergy, teachers, coaches, friends, therapists, and, in some cases, practitioners of alternative forms of medical care.

**Advanced Directives**

In the mid-1990s, Congress enacted the Patient Determination Act, which requires that all hospitals and health care institutions educate adult patients of their right to declare an advanced directive. An *advanced directive* is a declaration of a patient's wish for further care or the cessation of care in the event that they can no longer communicate or are incapacitated. An individual may be designated to speak on behalf of the patient. This decision facilitates health care decisions and reaffirms the patient's right to self-determination. In the practice of pediatric oncology, this issue directly affects adolescents older than 18 years of age. Although the law does not stipulate that children need to declare an advanced directive, many family members are already familiar with the standard and may actually expect to address this at the time of a hospitalization. For children younger than 18 years, the family must be involved and provide the directive for care. In discussing or answering questions concerning these issues, great attention must be paid to the cultural, ethnic, and religious practices of the family constellation, including distant relatives who might not share the same beliefs as the immediate family.

The psychological impact of discussing advanced directives with each hospitalization may be deleterious to the adolescent's state of mind. Often lacking self-esteem and confidence, the adolescent is particularly vulnerable and, in many cases, suspicious of medical providers. It is also a confusing time in which the hopes of therapeutic interventions intersect with the potential of treatment failure. In this regard, it is of particular importance to consider who will discuss the issue of advanced directives with the adolescent patient; the primary care team should address this issue and help the older adolescent to formulate a decision. Preparing him or her for this may eliminate some of the anxiety generated by the routine of asking patients at the time of admission. In many circumstances, in the discussion of advanced directives, adolescents may feel empowered and develop a stronger sense of self-worth.

The effect that advanced directives may have on the family's perception of care may influence the tenor and the compliance of proposed care, especially in preterminal cases. One could argue that because many of these issues are frequently on the minds of the patient and family with cancer, this may not be necessary. It takes a sensitive and knowledgeable physician or nurse to gauge the proper timing for addressing the issue of an advanced directive, especially with the adolescent. In a similar manner, addressing these issues with the family requires a keen understanding of the family's position on further therapy balanced against the prospect of losing further control and ultimately losing a child. Although the legal changes are not directly applicable to the pediatric setting, changes in the standard of care for adults will shape the family's view of many of the difficult issues raised in the terminal care of a pediatric or adolescent oncology patient.

**Death**

> “When doctors raise the issue of death, they're not the first to think of it. The patient and family have thought about it daily since the diagnosis. The docs are the last to get the nerve to bring it up. We don't talk about it with doctors because we're afraid it may jinx you. It could bring bad luck.”

Remarkably, death is a subject that is not often discussed. Who would more want to discuss death than someone who is facing it daily? When opportunities to talk about death arise, such as when another patient on the floor dies, do not ignore them. Patients and families may often give hints that they want to talk about death; it may be on their minds, but they will not necessarily raise the issue. Either nonverbal cues or pauses in a serious conversation may be all that is apparent. The subject may be gently introduced by an indirect question such as, “How are things going?” The response may be revealing and lead to questions of death and dying.

Most families realize when a patient on the ward is dying. They observe that the patient is moved to a single room, often in a special location that affords more privacy, that shades are drawn, or that there are changes in the number of visitors to a room. All of these rituals upset the tenuous sense of equilibrium on an oncology ward. Families fear this most of all and react strongly when they see others enduring it. If the physician and nurses do not acknowledge it, the families will find out from others on the floor. Families want to hear it from the medical staff, even if it is a simple recognition of the events down the hall. When it is not acknowledged, families may perceive that death is being avoided or “swept under the carpet.” This perception may also be interpreted as condescending to those who daily live in fear of death.

> “A friend asked, “How does leukemia feel—is it like a cold?” It sounds stupid but she was not so far off. Later, I was thinking what it would be like to die at age 20. I cried hard, but not for the jobs I would never have, nor for the money I was not going to make, nor even for the college degree I was never going to receive. On that day I thought of the many wonderful friends I had had and the chance that I would never see most of them again. I thought of my family, and it was life that I cried about. I thought that if I died that summer, I might never have lived. Yes, I thought, I felt cold.”

Many patients struggle with the images of what life will be like for those whom they leave behind. They may feel guilt for the sadness they are causing those around them, or they may feel great sadness because they will miss loved ones. Some children are afraid to talk about these issues, because they view their illness as punishment for past transgressions. Talking with patients about death, particularly if it is impending, requires great sensitivity to more than these issues. Helping a child to understand death requires insight into the cultural, religious, and philosophical beliefs of the family, as well as the fears and images engendered by the medical environment.

> “The events leading up to the death of my daughter are clear in my mind, 2½ years later. I remember the room number, the nurse and resident, the time of day, who was present, and what was said. Every other day falls in with the others except that one. I can see everything again, but with any other day, I can only think of a few important things.”

The date of a child's death will be forever imprinted in the parent's mind. How it happened and specifically what occurred, often in excruciating detail, are recalled over and over. Could anything have been done differently? Was everything done properly? Was my child comfortable? Did my child know we were there? What comes next, after the mechanics of a memorial service? Families will spend the rest of their lives wondering about the circumstances and reasons for the death.

The guidance and support offered by the pediatric oncologist helps shape how and what the family remembers. Although the death may be expected, the trauma and pain are not, no matter how much preparation is given. The task is to minimize the family's sense of responsibility for the death, no matter what the circumstances, and to reassure the family that everything possible was done on behalf of the child.

**After the Death of a Patient**

> “When our child died we were devastated. We felt like we lost everything. It was peaceful and calm when she died, but afterward came uncontrollable tears and sadness. When it came time for the funeral service, we noticed everyone who came. Our oncologist did not come. We were hurt and confused. He had been there through everything and knew her better than most, especially during the last year. He understood what was going on. When he didn't come, we felt let down. We thought for sure he'd be there.”

The decision to attend a funeral or memorial service of a patient is highly individual, based on emotional attachment and, often, commitments to other families. Whether the physician attends or not, however, parents appreciate hearing from the physician at this time. The recognition that it may be too emotionally painful for the physician to attend, especially if he or she felt grief over the loss, may be interpreted as a sign of compassion and care for the child. Furthermore, families view this as sympathy for their own tragic experience.

The physician may ask about the funeral arrangements and inform the family beforehand whether he or she can attend. This courtesy prevents disappointment and indicates that the physician cared enough to inquire and may have attended, had circumstances permitted.

> “I never thought I wanted to hear from the hospital again. I couldn't imagine going back, especially after his drawn-out death. But when the doctor called us at home about a month later, my heart raced as I picked up the phone. I didn't know what was going to happen, but when he invited us to come talk with him, I thought, How could I ever go back? Eventually, we saw him in his office, away from the clinic. Somehow, we both felt relieved and connected to our child. It helped

us to maintain the contact and to know that someone cared about all of us."

With rare exceptions, because of the special bond between physician and family, parents welcome follow-up contact. The opportunity to recount the events with many questions and search for an understanding of the specifics maintains a connection with those who witnessed the final events and confers a degree of finality.

**Litigation**

> "My 10-year-old son died of leukoencephalopathy 14 months after a bone marrow transplant for acute lymphoblastic leukemia when he was in complete remission. When we arrived at the transplant center I had expressed my concern about using intrathecal medications after the transplant, as I had read that leukoencephalopathy had occurred in as many as 20% of children in one study using such a protocol. I was told, however, by his oncologist at the transplant center that giving the intrathecal medication after the transplant would decrease his risk of a CNS [central nervous system] relapse. Even though he had never had a CNS relapse in suffering leukemia for 5 years, I trusted the doctor's decision. When my son developed leukoencephalopathy, I found out that most centers do not give intrathecal medications after transplant and only very rarely saw leukoencephalopathy develop in their patients. When I found this out, I just wanted to speak to the physician at the transplant center to talk things through, given my initial concerns. I had no intention of filing a lawsuit. He refused to return even my phone calls, and when I visited the transplant center, he refused to see me to discuss my son's situation. It was because of this failure to talk to me that I eventually filed the malpractice suit, which was settled out of court."

As unpleasant as the prospect is, in our litigious society, in a field such as pediatric oncology in which expectations for cure are increasing, whether realistic or not, there is always a risk of a malpractice suit if complications occur or even if everything possible was done and the child still dies. However, a major reason that a malpractice suit is filed against a physician is not necessarily medical negligence but failure to communicate. When the child is not doing well, it is particularly important to communicate to the parents that everything is being done. Naturally, any request by a parent for consultation should never be rejected, particularly if therapy has failed. Ignoring an uncomfortable situation will only antagonize already doubting parents, who may then pursue a malpractice suit.

## CONCLUSION

Cancer is unlike cystic fibrosis or mental retardation. Suddenly, a perfectly healthy, normal child is transformed into a seriously ill child who is fighting a life-threatening illness. If the patient survives the disease and its treatment, the patient will still have losses. Meanwhile, the entire family will continue to struggle to survive the trauma of childhood cancer. The surviving patient and family will spend the rest of their lives wondering how and why they were afflicted. There will always be that lingering concern that the cancer may return. It is the responsibility of the pediatric oncologist to address the care of the family throughout therapy because without the family's support, compliance is that much more difficult, and because it is the humane, compassionate, and caring thing to do.

Because pediatric oncologists treat cancer in children but, except in rare circumstances, do not experience it firsthand, it is most telling to hear what the patients and their families say. They are the teachers and leaders. We are their students.

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# ETHICAL CONSIDERATIONS IN PEDIATRIC ONCOLOGY

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## INTRODUCTION

What is the contribution of medical ethics to pediatric oncology? Certainly ethics is not like other medical subspecialties, which can be mastered only through years of training and clinical experience. We expect physicians to behave ethically regardless of their specialty, clinical experience, or training. Some might argue, therefore, that seeking ethical advice from a textbook chapter is tantamount to confessing that one is morally bankrupt.

However, as Charles Curran noted in the introduction to a chapter on ethics in another textbook, [1] medical ethics can be understood on at least two different levels. An analogy to psychiatry and mental health helps to explain the difference between the two. Many adults who have never read Freud or Jung are emotionally mature and well-balanced human beings. The practice of psychiatry is a second-order discipline, one that studies, orders, and categorizes the range of human behavior and emotion in a systematic way. By analogy, many adults are ignorant of Mill and Kant and yet still are models of moral integrity. The study of ethics is also a second-order discipline, an attempt to understand the components of moral behavior and to use this understanding to help to determine the best course of action when we are confronted with moral dilemmas. This chapter is an attempt to use this kind of systematic analysis to shed light on some of the ethical questions that arise in the practice of pediatric oncology.

The authors of this chapter have chosen to concentrate on a few important areas rather than cover the entire range of ethical concerns. These were chosen because they are areas that are of particular relevance and importance to clinicians who care for children with cancer. We begin with a discussion of informed consent and the ethics of human subjects research. This is followed by comments on confidentiality and genetic testing. Other sections of this text deal with palliative care in a general sense, so we have chosen to focus on end-of-life issues in the intensive care unit (ICU), as a number of ethical issues are unique to this setting. Finally, we look at financial incentives and conflicts of interest.

## INFORMED CONSENT

Just as recent advances in pediatric oncology have revolutionized the treatment of childhood cancer, so the concept of informed consent has altered the traditional approach to medical decision making. From the times of Hippocrates until the recent past, medical decisions had been based almost entirely on the opinions and preferences of the physician. Today, the locus of authority for these decisions has shifted dramatically away from the physician to the patient. Although the former approach now carries the pejorative label of “paternalism,” these traditional practices should not reflexively be judged as necessarily unethical. For the most part, physicians always have acted in good faith to promote the health and welfare of their patients as they perceived them. Although this traditional approach no longer is acceptable as a guide to practice, some respected bioethicists have maintained that the pendulum has, in fact, swung too far in the other direction and that this older and more beneficent approach to the practice of medicine and the patient-physician relationship has many positive features that we should try to preserve. [2]

The modern approach to informed consent has resulted from the confluence of distinct trends in the law, bioethics, and society. Although legal cases involving questions of informed consent can be traced back to the 1700s, perhaps the clearest legal statement of the concept was articulated in 1914 by Justice Cardozo: “Every human being of adult years and sound mind has the right to determine what shall be done with his own body.” [3] In the years since, a long series of legal cases have given specificity and substance to how this right should be defined within the context of medical decision making. This trend in the law has been paralleled by similar developments in the field of bioethics, in which respect for patient autonomy has become recognized as a dominant ethical principle. Finally, each of these trends has in fact been a reflection of societal forces that increasingly have emphasized the importance of individual rights. These have manifested in the Western world as a variety of “rights” movements (women's rights, civil rights, gay rights, etc.), and internationally as a movement for human rights more generally and for democratic reform.

What is the relationship between this broad historical perspective and the practice of pediatric oncology? What is the relationship, for example, between the societal struggle for political rights and decision making for a 5-year-old child with cancer? To make sense of this relationship, we must follow the progression of the informed consent doctrine as it has evolved, first with regard to competent adults, then to incompetent adults, and finally to children.

### Informed Consent for Competent Adults

The doctrine of informed consent is most straightforward and least ambiguous when applied to individuals “of adult years and sound mind.” Various commentators have defined five criteria that together constitute valid, informed consent: (a) competency, (b) disclosure, (c) understanding, (d) voluntariness, and (e) decision. [4]

### *Competency*

The core meaning of *competency* is “the ability to perform a task.” Within the professional jurisdictions of law, psychiatry, and philosophy, however, competing definitions that have been developed are not mutually consistent. This is a particular problem in pediatrics, in which the law makes a general assumption that patients under the age of majority are not competent to make decisions for themselves. To minimize the confusion related to these multiple definitions, it is helpful to avoid use of the term *competency* outside of this legal context and to focus on the concept of decision-making capacity as the relevant factor in regard to informed consent. Decision-making capacity can be judged in relation to three parameters: (a) patients' ability to understand the medical situation and the alternative choices under consideration, (b) patients' ability to reason about and to appreciate the consequences of the available choices, and (c) patients' ability to make a decision between the available choices, preferably on the basis of a coherent set of values and preferences. Patients who have decision-making capacity should be included in the process of informed consent, regardless of whether they are competent in the legal sense (e.g., mature adolescents).

### *Disclosure*

Disclosure refers to the information that must be communicated from the clinician to the patient before consent. This information includes (a) the nature and purpose of the proposed treatment, (b) the foreseeable risks and discomforts, (c) the potential benefits, and (d) the available alternatives. Many clinicians take a cynical view of this requirement, as illustrated by the comment, “If the patient wants to know that much information, they should go to medical school!” Any reasonable interpretation of this requirement, however, recognizes that clinicians must be selective in the amount and type of information they provide.

Three standards have been developed to guide physicians in selecting the information they present to patients. Two of these standards have a legal basis. Under the professional standard, the physician has a duty to provide the information that a reasonable physician would provide in similar circumstances. Under the reasonable-person standard, the physician must provide all the information that is material to the decision (i.e., all the information that a reasonable decision maker would want to know). A third standard, the subjective standard, is an ethical ideal that has no specific correlate in the law. Under this standard, the clinician should provide all the information that is material not just for a reasonable person but for *this particular* person. For example, physicians using this approach would draw on their unique knowledge of the patient—the patient's preferences, fears, life story, family history, values, and the like—to tailor the information to best meet the patient's needs.

As a general rule, common complications should be disclosed regardless of severity, and risks that are serious or irreversible should be disclosed regardless of frequency. This rule of thumb would advise that clinicians always mention death as a possible complication, even when the risk of a fatal outcome is slight. This is perhaps prudent from a legal perspective, but we believe that the subjective standard can serve as a useful guide in this regard: Patients who present themselves as “wanting to know everything” generally should be told about the risk of death, whereas those who are looking to their clinicians for reassurance may be spared this disclosure unless it is truly a material risk.

### *Understanding*

It is possible to have objective knowledge of what information is disclosed, but it is not possible to have objective knowledge of what information the patient has understood. Hence, although the ethical requirement for understanding should take precedence over the ethical requirement for disclosure, most of the emphasis in the law has been on the latter, primarily because it can be assessed more objectively.

Through the process of shared decision making, however, clinicians should be engaged constantly in assessing the degree to which patients understand the diagnostic and treatment alternatives. This can be particularly difficult around choices involving risk. Studies have shown, for example, that individuals will choose different treatments, depending on whether the risks are presented as the probability of success or the probability of failure. [5] This psychological dynamic could have powerful consequences in the practice of pediatric oncology if, for example, a clinician optimistically presents one option in terms of its chances for clinical remission while presenting the alternatives in terms of their probability of tumor recurrence.

### *Voluntariness*

The free choice of patients can be influenced by persuasion, coercion, or manipulation. Physicians may at times use persuasion as an ethically appropriate strategy in the process of obtaining informed consent. If, for example, parents refuse initiation of chemotherapy for a potentially curable malignancy because of their concerns over the side effects of treatment, clinicians should not hesitate to challenge the parents and to question whether they are rationally balancing the short-term adverse effects against the long-term probability of survival. Indeed, failure to attempt to persuade parents in these circumstances would be ethically inappropriate.

Coercion involves influencing patients' decisions through the use of a credible threat. For example, a clinician may tell parents that if they refuse to consent to chemotherapy, the hospital attorneys will seek a court order to initiate treatment without their consent. Although threats of this type occasionally may be necessary and appropriate, they should never be used except as a measure of last resort.

Manipulation occurs when clinicians alter the process of disclosure in a way that emphasizes or minimizes some of the information relevant to the decision. Rather than trying to persuade patients after fairly presenting all the facts, clinicians may attempt to influence decisions by a selective presentation of the facts. Although perhaps well intended, this approach would fail to meet the standards of voluntary informed consent.

### *Decision*

Many clinicians see the goal of informed consent as obtaining a signature on the bottom of a form. The verb *consenting* recently has emerged in hospital jargon to describe this activity. However, the perceived legal need to have a signed form unwittingly has mislead many clinicians into conceptualizing informed consent as an event rather than a process. [6] Particularly in the practice of pediatric oncology, in which patients and families generally are faced with multiple decision points that must be navigated independently on the basis of a patient's evolving clinical condition, clinicians should recognize that obtaining a signature on a form is only the beginning of the process of informed consent.

## Informed Consent for Incompetent Adults

The model of autonomous decision making already described has been such a powerful paradigm for defining the process of informed consent that it has overwhelmed any other possible models of decision making. Rather than assuming that because patients are not autonomous, they therefore do not have the right to make their own decisions, the law has sought to define ways in which these rights can be exercised by others.

Two standards have evolved to guide surrogates who must make decisions for patients who cannot decide for themselves. [4] The *substituted-judgment standard* seeks to make decisions on the basis of the actual values and preferences that such patients had before becoming incompetent. As articulated by the court in Saikewicz, the decision “should be that which would be made by the incompetent person, if that person were competent, but taking into account the present and future incompetency of the individual as one of the factors which would necessarily enter into the decision making process of the competent person.” [7] The somewhat tortured language of the court in this case illustrates the extent to which the courts have bent over backward to base medical decision making on the autonomous choice of affected individuals.

The goal of basing decisions on the actual wishes of patients has given rise to efforts to have patients articulate these wishes beforehand through the use of advance directives. One type of advance directive, the living will, allows patients to specify the extent to which they would like to have life-sustaining medical treatments provided should they develop specific medical problems while incompetent. [8] The Patient Self-Determination Act, passed in 1990, requires hospitals to inquire whether patients currently have an advance directive and gives them an opportunity to create one if they do not. [9]

Obviously, the substituted-judgment standard cannot be applied directly to patients who never have been competent, because they never have had the opportunity to develop specific preferences about the care they would like to receive. The ethical standard used in these circumstances is the *best-interests standard*. This standard requires surrogates to make medical decisions that are most in accord with affected patients' best interests. Although this principle provides useful guidance for

surrogates, it does not resolve situations in which views are competing about how best to advance the interests of involved patients.

## Informed Consent for Children

Ethical issues in informed consent for children often are analyzed under the category of the noncompetent patient, yet children differ from noncompetent adults in many important ways. For example, most of the sentinel legal cases involving noncompetent adults have dealt with patients who never were expected to regain competency (i.e., adults with chronic and usually progressive medical problems). Children are different, because in most cases their competency and decision-making capacity are in a state of growth and evolution. With adults, therefore, we strive to respect their *former* autonomy; with children, the challenge is to preserve options faithfully for their *future* autonomy.

### *Children Unable to Participate in Decision Making*

From the newborn period through early childhood, children obviously are not able to participate in decisions about their medical care. During this time, parents generally are viewed as their surrogate decision makers. Up until the last century or so, children were seen essentially as the property of their parents, and parents were seen as having a right to make these decisions. Although this no longer is the case, the presumption in favor of parental decision making is based on several persuasive considerations: (a) Parents have strong emotional bonds to their children and are motivated powerfully to make decisions that are in the best interests of their children; (b) we presume that children will grow up to espouse many of the same values as their parents; therefore, parental decisions are more likely to resemble the kinds of decisions that children will make when they become competent; (c) parents usually will have to shoulder and live with the consequences of the decisions that are made on behalf of their children (including financial obligations), so they should have some say in making those decisions; and (d) parents are held responsible for most of the nonmedical decisions that must be made on behalf of their children (housing, food, schooling, etc.), so they should have responsibility for the medical decisions as well.

### *Children Able to Assent to Medical Treatment*

The concept of assent to treatment for pediatric patients was first proposed by the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research in the 1970s.[10] On the basis of knowledge of normal childhood development, this commission proposed that children between the ages of 7 and 14 should be asked for their assent to medical treatment. After the age of 14, they suggested, children generally should be presumed to have full decision-making capacity. The American Academy of Pediatrics sanctioned this approach in 1995 and further claimed that the entire “doctrine of 'informed consent' has only limited *direct* application in pediatrics. Only **patients** who have appropriate decisional capacity and legal empowerment can give their **informed consent** to medical care. In all other situations, parents or their surrogates provide **informed permission** for diagnosis and treatment of children with the **assent** of the child whenever appropriate” (bold and italics in original).[11]

What is the relevance of this concept in pediatric oncology? In most cases, childhood cancer is a life-threatening situation, and even the experimental treatment protocols are driven more by an intent to provide therapy than to perform research. In most instances, therefore, treatment for pediatric cancer will not be contingent on obtaining the assent of affected children. As the American Academy of Pediatrics was careful to point out, however, in situations wherein such children will have to receive medical care despite their objection, they should be told of that fact and not be deceived.

### *Emancipated and Mature Minors*

Two legal categories that give special status to patients under the age of majority also must be mentioned.[12] Emancipated minors fall into a legal category that grants certain individuals under the age of majority all the rights of adults to consent to medical care. State laws vary, but most specify by statute the conditions under which a minor is considered emancipated. Generally, minors are emancipated when they are married, are parents, or are on active duty in the armed forces. In some jurisdictions, minors are emancipated when they are beyond a certain age (e.g., 16 years), are not financially supported by their parents, and either are not subject to parental control or their emancipation has their parents' consent. (Therefore, runaways would not generally be considered emancipated.)

Many states have either statutory or case law for the treatment of mature minors. Mature minors are not emancipated, but nevertheless, they may have the legal power to consent to some forms of medical treatment. Morrissey et al.[13] suggested criteria for finding a minor's consent sufficient to authorize treatment ( Table 48-1). Although the mature-minor concept provides legal protection to physicians who treat adolescents, such patients' parents are not financially responsible for treatment rendered without their consent.

1. The minor is 15 yr of age or older.
2. The minor can give informed consent; that is, in the judgment of the treating physician, the minor appears to understand and appreciate the benefits and risks of the proposed treatment and can make a reasoned decision based on such knowledge.
3. The proposed treatment is for the minor's benefit and not for the benefit of another.
4. The proposed treatment is deemed necessary according to best professional judgment.
5. The treatment does not involve complex, high-risk medical procedures or complex, high-risk surgery.

Adapted from Morrissey JM, Hofmann AD, Thrope JC. Consent and confidentiality in the health care of children and adolescents: a legal guide. New York: Free Press, 1986.

**TABLE 48-1. CRITERIA FOR FINDING A MINOR'S CONSENT SUFFICIENT TO AUTHORIZE TREATMENT**

## Ethical Dilemmas about Informed Consent in Pediatric Oncology

### *What If the Parents Do Not Want Their Child to Know the Diagnosis?*

Several decades ago, adult patients with cancer frequently were not told of their diagnosis; similarly, the early literature in pediatric oncology also supported the view that it was unethical to inform children with leukemia of their diagnosis, as they needed to be protected from the psychological harms of knowing that they had a life-threatening illness. By the late 1970s, however, cultural changes had led to a reversal of this view, with recommendations for full disclosure to adults and disclosure as appropriate to children.[14]

In addition, there may be psychological advantages to children from early disclosure of their diagnosis. Data from a retrospective study by Slavin et al.[15] suggested that survivors who learn of their diagnosis early may have a better psychosocial adjustment than those learning late. In that study, many of the parents who initially did not share the diagnosis with their child felt that this created a source of stress both during and after the treatment period. Some of these children who learned of their diagnosis late were reported to have greater difficulty in integrating the information, and some were described as feeling betrayed. Although this study found statistically significant differences in psychosocial adjustment between the “early” and “late” learners, it could not establish a causal link between these factors, because possibly early learners came from families with a more open style of communication that may have improved their outcomes independently.

### *What If the Child Refuses Necessary Diagnostic or Therapeutic Procedures?*

Just as adolescents may have the capacity to participate in their decision making, they are also well known to have the capacity for (what most adults regard as) irrational behavior. Billy Best, for example, was a 16-year-old patient with stage II Hodgkin's disease diagnosed in 1994. He and his parents were told that he had an 80% to 90% chance of cure with chemotherapy and low-dose irradiation. Although he reportedly had only minor side effects from the chemotherapy (including hair loss, nausea, and fatigue), after 2.5 months he refused treatment and ran away to Texas from his Massachusetts home. He returned only on the condition that he would not be forced to undergo additional treatment. Negotiations with a team of clinicians at the Dana-Farber Cancer Institute in Boston failed to persuade him or his

parents to continue with treatment. Ironically, Billy and his family did travel to Canada to receive alternative therapy, part of which involved multiple self-administered injections of an unproven compound containing camphor, nitrogen, and organic salts. Eventually, his clinical team chose neither to sever their clinical relationship with him nor to seek legal action in an effort to impose proven therapy. Rather, they entered into a signed agreement with him and his family to respect his refusal of treatment while still monitoring him for evidence of cancer. [16],[17] and [18]

Clinicians and parents have not always refrained from imposing standard treatment, however. In New York in 1991, for example, a 15-year-old boy was given a diagnosis of anterior mediastinal tumor. The patient's father had died of carcinoma of the lung 4 months earlier. Largely on the basis of his phobia of needles, the patient refused to undergo diagnostic surgery. His mother asked the court for an order directing the child to submit to surgery. The court found that surgery was urgently required and ordered the sheriff's department to take him to the hospital, to restrain him if necessary, and to supervise him while he was in the hospital. [19]

These two cases illustrate the kinds of problems that arise in the gray area of late adolescence, in which patients do not yet have the nearly unqualified rights of adults to refuse medical therapy, yet parents no longer have authority to mandate their children's treatment. The best recommendation that can be made is for clinicians to attempt to persuade adolescents regarding the optimal approach to their care. When these recommendations are refused, however, clinicians must decide whether this decision is reasonable, all things considered, or whether it is in the patient's best interest to seek a court order imposing the institution of standard therapy. When in doubt, the bias should be toward potentially life-prolonging treatment, as this path is least likely to foreclose options for patients as they mature into fully functioning autonomous adults.

### *What If the Parents Refuse Necessary Diagnostic or Therapeutic Procedures?*

Western allopathic approaches to medicine no longer enjoy an unchallenged dominance in many segments of society. Families from different cultures may not approach health care decision making and treatments with the same assumptions as those of allopathic practitioners. In addition, the alternative medicine movement is a rapidly growing combination of approaches that may be adopted by patients and families either exclusively or in combination with allopathic treatments. [20]

Recently, a Korean family brought their 2-year-old child to a children's hospital for management of Ewing's sarcoma of the proximal femur. Standard management included chemotherapy followed by either radiotherapy or surgical amputation, with a predicted 5-year survival of 60% to 80%. The family agreed to treatment but, after the child received the chemotherapy, the family refused to go forward with either radiotherapy or amputation. They understood that either of these would leave the child with a severe cosmetic deformity of her leg. They planned on returning to Korea within the next several years and explained that Korean views about disability were very different from those in the United States. They believed that with the expected degree of disfigurement, their daughter would be a social outcast. Although survival rates with chemotherapy treatment alone were unknown (as this treatment strategy had never been offered), they were thought to be substantially lower than rates with standard treatment. Nevertheless, the family believed that the additional increment in survival was not sufficient to justify the physical deformity. In meetings between the parents and clinicians, the parents implied that if they felt pressured to change their mind, they immediately would return home to Korea.

Cases such as these raise many questions that are not answered easily. Should cultural differences matter? Even if they do, how much of parents' views can be attributed to differences between cultures and how much can be ascribed to idiosyncratic or even irrational opinion? One of the hallmarks of good ethical decision making is that similar cases should be decided similarly: If the clinicians would seek a court order to treat a white child in similar circumstances, what arguments could justify not seeking a court order for a Korean child?

In many areas of pediatric practice, clinicians have a relatively low threshold for overriding the decisions of parents when they refuse prescribed therapy on religious or cultural grounds. For example, clinicians obtain court orders to transfuse blood to the children of Jehovah's Witness parents on an almost routine basis. Unlike conditions that require a blood transfusion (for which treatment can be completed within a matter of hours), cancer is a chronic disease that is treated over a period of months or years. For this reason (among others), pediatric oncologists should place an especially high priority on trying to maintain working relationships with families. This may mean making concessions to family demands that are not consistent with optimal medical management but do not place the child in great peril either. These concessions may be necessary to maintain a family's cooperation with the administration of medications, the keeping of outpatient appointments, and the like. In many cases, the only alternative to this plan will be to place the child in foster care for the duration of the treatment, accepting the disruption of the family and the psychological trauma that this will entail.

## HUMAN SUBJECTS RESEARCH

One of the most remarkable features of pediatric oncology is the widespread use of clinical trials to study the efficacy of treatment regimens. Perhaps no other specialty in medicine has as many of its patients enrolled in research protocols. Although this effort certainly is to be commended, one potential concern with this practice is that the ethical boundaries and distinctions between clinical care and research may become blurred.

The ethical conflict that physicians experience between their role as clinician and their role as scientific investigator is profound. As Schafer put it, “In his traditional role of healer, the physician's commitment is exclusively to his patient. By contrast, in his modern role of scientific investigator, the physician engaged in medical research or experimentation has a commitment to promote the acquisition of scientific knowledge.” [21] This potential conflict has motivated the development of several important codes to promote the protection of research subjects (summarized in Table 48-2).

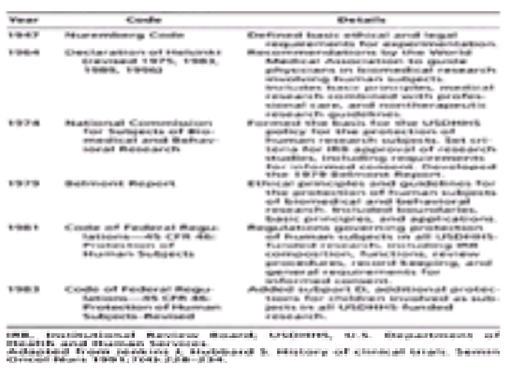

**TABLE 48-2. LANDMARKS IN THE PROTECTION OF CHILDREN AS RESEARCH SUBJECTS**

### Concept of Clinical Equipoise

The roles of clinician and investigator can come into conflict when physicians randomly distribute patients between the control and treatment arms of a study. Some have argued that it would be unethical for physicians thus to assign patients unless such physicians had absolutely no preference for one treatment over the other (i.e., unless the physician was in “personal equipoise”). Others have recognized that this standard is unreasonably strict and that it is ethical for a physician randomly to assign patients between treatment arms as long as a subgroup within the medical community as a whole prefers the alternative treatment. In other words, when the chosen treatment for any particular patient depends primarily on the physician they happen to see, the medical community is in a state of clinical equipoise, and randomization between the two treatment arms in a clinical trial is not considered a violation of a physician's clinical duties to the patient. [22] Clinical equipoise, therefore, is an absolute requirement for the conduct of clinical research that compares the efficacy of two treatments or of a treatment against placebo.

The concept of clinical equipoise does not resolve all the problems in the conflict between the roles of clinician and investigator, however. First, it assumes that because of genuine uncertainty within the medical community regarding the optimal treatment for a condition, patients will be satisfied with having their therapy determined by a flip of the coin. In fact, patients generally expect physicians to integrate the consensus views within medicine into overall recommendations that include both personal experience and clinical judgment. To this extent, patients enrolled in studies may not receive some aspects of the personalized care that physicians typically render when they are acting purely within their role as clinician.

Second, just because clinical equipoise may exist within the medical community, it does not necessarily mean that affected patients are in equipoise with regard to treatment preference. Imagine a trial between chemotherapy and radiotherapy for treatment of Hodgkin's disease: Even if the clinical community is uncertain about which of these treatments offers the best long-term survival, patients may have strong preferences for one treatment over the other on the basis of additional factors, such as side effects and other sequelae.

Finally, clinical trials generally are designed to terminate when one treatment has been shown to be statistically superior to the other, with a probability value of £.05. Yet, well before this threshold is reached, there must be a trend in the data in the direction of the treatment that is proving to be more successful. Neither patients nor their treating physicians are allowed to see these data, however, as they could influence their willingness to enroll in the trial and thereby jeopardize the chances of the trial's reaching a conclusion. Keeping the data secret in this way clearly is important for the conduct of good research, yet it cannot be justified from the perspective of an affected patient, who desires to have all the available information as part of the decision-making process. In sum, the conduct of good clinical research has the potential to threaten the fiduciary responsibilities of physicians to their patients, even when the requirement for clinical equipoise is met.

**Special Requirements for Research with Children**

From 1973 to 1978, the National Commission for Subjects of Biomedical and Behavioral Research published a series of reports that form the basis for the current regulations regarding the conduct of human subjects research. These included a number of special requirements for children. [10,23]

First, the commission developed the concepts of assent and permission already discussed. These concepts take on a much more powerful meaning in the context of clinical research, because here the assent of affected patients not only is ethically preferable but often is required. In addition, some children who are not developmentally capable of assent still may be capable of "deliberate objection." For example, a 4-year-old incapable of providing assent may, nevertheless, be able to protest, "No, I don't want to be stuck with a needle." This would be considered an expression of deliberate objection. On the other hand, infants' nonspecific crying or withdrawal from a variety of stimuli would not be regarded as an act of deliberate objection. Unless research has the prospect of benefit to affected children, the lack of assent or a deliberate objection should be taken as a veto of such children's participation in the research. [23] As noted, however, the life-threatening nature of childhood cancer and the therapeutic intent of the treatment protocols generally will mean that the experimental treatment for children with cancer will not be contingent on such patients' assent.

How does the concept of *assent* differ from that of *informed consent*? The existing regulations are not explicit about this question, but the difference probably should be construed as a matter of degree. As Levine noted, "It may be appropriate to provide some children with 'a description of any reasonably foreseeable risks or discomforts,' without providing 'an explanation as to whether any compensation . . . (is) . . . available if injury occurs.' " [23] A comparison of the elements of informed consent and the elements of assent is presented in Table 48-3.

**TABLE 48-3. ELEMENTS OF INFORMED CONSENT AND ASSENT**

In addition, the commission differentiated between various degrees of risk presented by the research and whether the research held out the prospect of direct benefit for involved patients. Degree of risk was stratified in terms of (a) minimal risk, (b) a minor increment above minimal risk, and (c) more than a minor increment above minimal risk. *Minimal risk* is defined as "the probability and magnitude of physical or psychological harm that is normally encountered in the daily lives, or in the routine medical or psychological examination, of healthy children." Research that does not hold out the prospect of direct benefit for affected children generally should involve no more than minimal risk.

Research that may not benefit such children but involves greater-than-minimal risk can be justified only under certain narrow circumstances. First, the procedures must be those that such children normally might experience by virtue of their disease or medical condition. For example, it might be appropriate to ask children who have leukemia and have had several bone marrow examinations to consider having another for research purposes. Because of their experiences, these children would be expected to be familiar with the procedure and its attendant discomforts and thus would be in a knowledgeable position to make the decision. Another requirement is that "[t]he anticipated knowledge is of vital importance for understanding or amelioration of the subject's disorder or condition . . ." (Department of Health and Human Services Rules and Regulations, 45 CFR 46). This requirement reflects the presumption that children should undergo research involving more than minimal risk only when the knowledge to be obtained is of direct relevance to diseases or conditions present in such children.

When research has the prospect of providing direct benefit for affected children, the strict standards regarding both risk and the need for assent are revised. With regard to risk, the research is justified if the risk is proportionate to the anticipated benefits and if the risk-benefit ratio for the research is at least as favorable as that presented by any alternative approaches. In addition, the assent of such children is not a necessary condition when the research may benefit the children and this benefit is available only in the context of the research.

**Phase I Trials in Pediatric Oncology**

In general, studies should be conducted first on animals, followed by adult humans. Older children should be the subjects of trials prior to those involving infants. This approach reflects our concern for justice and the view that the most vulnerable members of society should be the most protected from the burdens of research. In situations wherein a disease or condition occurs only in children, however, an important consideration is that these concerns should not stifle the development of successful therapies. For certain pediatric malignancies, genetic diseases, and conditions, such as pediatric human immunodeficiency virus, phase I trials are increasingly being performed.

Another reason to perform phase I trials in children is that the pharmacokinetics of drugs may differ substantially from those in the adult population. Indeed, the reluctance to perform rigorous drug testing in children has been a disservice to child health overall. Although it is true that medications proven safe and effective for adults usually do not produce adverse reactions in children, when they do, the number of children harmed is much greater than if the drugs had been studied systematically at the outset. Examples include the "gray baby" syndrome with chloramphenicol, permanent staining of developing teeth with tetracyclines, and phocomelia with the administration of thalidomide to pregnant women. Indeed, the U.S. Food and Drug Administration recently took a strong advocacy position on the need to include children in pharmacologic research.

Despite these reasons supporting the need to enroll children in clinical trials, the ethics of enrolling children in phase I trials remains controversial. [24] The primary objectives of phase I trials are to determine the maximum tolerable dose (MTD) of the drug and to develop a preliminary profile of its toxicities. The first group of research subjects receives a dose unlikely to be either toxic or beneficial, and the dose is increased with each subsequent group until the MTD is identified. As all these studies clearly involve more than minimal risk to the patient, enrollment of children in these studies is ethical only if the trial can be said to have a therapeutic intent. More specifically, current regulations require both that the risks are proportionate to the intended benefits and that the risk-benefit ratio of the chemotherapy is at least as favorable as the alternatives.

Whether phase I research in children fulfills these requirements is debatable. On one hand, children generally are spared the possibility of undertreatment in these

trials, because pediatric phase I trials generally start at 80% of the MTD determined in adult trials. [25] On the other hand, even with this advantage, the likelihood of benefit still is very low. On the basis of data from 577 pediatric patients enrolled in phase I trials between 1967 and 1988, only 34 (6%) objective responses (both partial and complete) occurred.[26] The duration of the 11 complete responses ranged from 12 to 300 days, with a median of 60 days. These data are similar to those obtained in adults: A study of 6,447 adult patients entered into phase I trials found a 4% objective response rate overall.

This possibility of therapeutic benefit must be balanced against the risks of harm. In the foregoing pediatric study, the deaths of 3% of the patients who died were attributed to side effects of the phase I agents. In addition, generally nonlethal complications arise from these agents, including infection, bleeding, renal and hepatic dysfunction, and nausea and vomiting. The added pain and discomfort of the diagnostic procedures that must be performed to monitor the effects of the medications also must be considered. Finally, participation in a phase I trial may mean that affected children cannot enter into a palliative care program that would focus exclusively on such children's comfort and quality of life. As a result, these children might eventually spend in the hospital time that otherwise would have been spent at home during the last days of their lives.[24]

On the basis of this analysis of the risks and benefits of participating in phase I trials, we conclude that the decision of whether to participate should be based on the considered judgment of affected children and their families, in the context of all their hopes, fears, values, and goals. This conclusion must be tempered, however, by evidence that patients and families faced with this decision often seem to base their choice on factors that may seem irrational to a nonbiased observer. This seeming error in judgment on the part of patients and families considering participation in phase I trials has been labeled the *therapeutic misconception*.[27]

For example, in one small adult study that examined the motivations of patients to participate in phase I trials, a majority (85%) decided to participate for reasons of possible therapeutic benefit. A small number of patients (11%) decided to participate in a phase I trial because of advice or trust in a physician or because of family pressures (4%). No identifiable altruistic reasons were given for participating in a phase I trial. [28] With regard to the question of whether patients consider the option of palliative care rather than participation in a phase I trial, 44% of the patients in this study said that no other options besides participation in a phase I trial were discussed, and only 30% of patients acknowledged that the option of focusing only on supportive care was discussed.

Phase I trials, therefore, present a difficult dilemma for researchers in pediatric oncology. Certainly, patients and families who choose to enroll in these trials are able to maintain some hope of remission or at least a partial response. Investigators have an obligation, however, to be sure that this choice is as fully informed as possible and is not purely an expression of desperation when more viable therapeutic alternatives have been exhausted.

### Phase II Trials in Pediatric Oncology

The purpose of phase II trials is to demonstrate effectiveness and relative safety of new medications. Because phase II trials involve medications that already have been shown to have some effect, the question of therapeutic intent is less controversial than in phase I trials. The ethical questions in these trials center more around whether children are fully informed about the nature of the research and are apprised adequately of the alternatives to participation.

Nitschke et al.[29] reported an innovative approach toward enrolling pediatric patients in phase II trials that, although published in 1982, still presents an interesting and challenging alternative to current practice. In that study, patients older than 5 years engaged in a process of choosing between participation in phase II research and supportive care. Of 44 families asked to participate, only 1 refused. Of these 43 families, the parents were offered the alternatives of having the physician explain the choice between supportive care and the phase II study to the child in a family meeting and of having the parents explain the choice to the child privately after their discussion with the physician. The group conference with the physician was chosen by 36 parents and the private conversation by the remaining 7. The authors reported that “[t]he majority of the children made the decision,” with 14 choosing the phase II trial and 28 choosing supportive care (1 adolescent did not make a choice and was lost to follow-up). The information communicated by the physician in discussing the options turned out to be a controversial aspect of the study. Initially, the clinicians included mention of benefits to medicine and to future patients as one aspect of participation in the phase II study. After one noncompliant patient mentioned that he wanted to refuse therapy but did not do so because he thought his physicians desired to know more about the drug, this perspective on potential benefit was dropped from the discussion.

This study supports the importance of providing the full menu of options to children who are considered as subjects of phase II trials, while recognizing that clinicians must be careful not to bias children's decisions by subtly favoring one approach over the other. The study also demonstrates that, when fully informed of available alternatives, many children will choose supportive care over participation in research.

### Disclosure of Trial Results to Patients and Families

Patients and families who have participated in clinical trials generally are not informed about the results of those trials after completion of the studies. In recent years, however, subjects of trials have become more involved and have desired a greater degree of participation in the research being performed. In addition, subjects have expressed an interest in knowing the results of the trials in which they have participated.

Snowdon et al.[30] reported on parents' reactions to receiving the results from a randomized controlled trial comparing the use of extracorporeal membrane oxygenation against standard therapy for neonates with life-threatening respiratory failure. The decision to give the parents of enrolled children the option of receiving the trial results was made at an early stage in the design of the study. The approach differed, depending on whether the newborn had survived. Parents of surviving children were sent a questionnaire about their experiences and asked whether they wanted to know the results of the trial. Seventy-five percent of the questionnaires mailed to surviving infants were returned, and all requested a copy of the findings. For the parents of those who had died, the questionnaire was mailed only after first determining from the pediatrician whether contact with the family was appropriate. Of those who were mailed the questionnaires, 57% responded, and 8% specifically declined the invitation to receive a copy of the results. Detailed interviews were performed with the parents of 24 of the surviving newborns. Although the responses of these parents to receiving the results generally were positive, some parents of children who were assigned randomly to the control group were upset by the findings, even though their children survived. The study did not address the responses of those who requested the results after their children had died.

Limited conclusions can be drawn from this one study. If feedback of results to the study participants is contemplated, plans for this should be incorporated into the design at the start of the trial. In addition, results should be sent only to those who respond positively to the offer. Finally, clinicians must consider that patients or families who discover that they were selected randomly to the less successful treatment may experience feelings of anger and confusion, even if they turned out to have a good outcome.

## CONFIDENTIALITY

Confidentiality always has been a central aspect of medical care. The Hippocratic Oath has physicians pledge that “[t]hings I may see or hear in the course of the treatment, or even outside of treatment regarding the life of human beings . . . I will never divulge, holding such things unutterable.” Indeed, some have argued that every culture has a requirement for its health care providers to maintain patient confidentiality. Today, concern is growing over confidentiality. To some degree, this concern is fueled by the growth of electronic medical records and databases that allow the exchange of patient information to more people, at great distances, with little effort. Another source of concern is the selling of patient information for commercial purposes, whether it is pharmacies' sales to drug companies of names of patients who have specific prescriptions or Internet health site sales of identities to marketers. Similarly, the public's suspicion of insurers and managed care companies has raised concerns about how they are using patient information and whether they are using this information to avoid high-cost patients. Finally, advances in genetics and the sense that genetic knowledge is a type of more intimate personal knowledge that could be used for employment discrimination and loss of insurance coverage also has caused people to be more concerned about confidentiality.

The traditional ethical standard of confidentiality is well stated by the Hippocratic Oath: Patient information is privileged, and physicians should not disclose patient information without affected patient's consent—or, for children, a patient's guardian's consent. This standard is justified by many considerations. The first is an intrinsic reason: respect for persons. Confidentiality recognizes that privacy is essential to being a free, autonomous person; privacy is a fundamental aspect of human dignity and of the ability to form relationships.[4,31] Thus, control over what is revealed about oneself, to whom, and under what circumstances is integral to being human. A further instrumental reason applies. It is argued that people will be less likely to seek medical attention and reveal important health-related information to health care providers unless information is kept confidential; this can have significant consequences not just for an affected person but, as regards communicable diseases, for other people.[4] Finally, confidentiality of health information is a widespread social expectation that defines norms of acceptable practice.

Two major issues complicate this general norm about confidentiality. First, patient confidentiality in pediatric care is complicated because parents and guardians are

involved intimately in medical decisions, rendering them entitled to information about the minors for whom they are responsible. However, sometimes it may be appropriate to keep patient information confidential even from parents and guardians. Second, despite being viewed as both intrinsically and instrumentally important, confidentiality of medical information is not absolute.[4] Myriad legitimate reasons substantiate that patient confidentiality can and should be breached.[32]

Providing specific advice about how these complications are to be addressed is difficult, because determinations often depend on the details of particular cases and because current legal standards of confidentiality vary from state to state. (The U.S. Department of Health and Human Services currently is promulgating new regulations about confidentiality, but these apply only to electronic records.) General guidance regarding withholding information from parents and guardians relates to situations in which such disclosures might harm affected patients. Thus, many states permit the withholding of medical information about a pediatric patient from parents and guardians if the information is related to (a) sexual activity, pregnancy, and abortion; (b) treatment for alcohol and drug abuse; (c) psychiatric treatment; and (d) treatment for communicable diseases, especially sexually transmitted diseases. Obviously, these exceptions can apply only to adolescents and mature minors who may be capable of making their own decisions. In such cases, physicians should assess the circumstances and, when appropriate, encourage adolescents to discuss these situations with their parents or guardians, providing support and counseling in the process.

In general, patient confidentiality can be breached for three broad reasons: avoiding harm to others, benefiting the patient, and public health reporting. In the Tarasoff case, breaching patient confidentiality was deemed ethical and legal when the patient presented a credible threat to the well-being or life of another person.[4,33] Further, some states mandate breaches of confidentiality by health providers in cases of child abuse and neglect, justifying this practice in the belief that such disclosures ultimately will be for the patient's best interest. Similarly, revealing information to consultants is justified by the benefits to the patient. Public health laws also may require reporting a variety of information ranging from communicable diseases to the provision of certain interventions.[32] Finally, it is important to recognize that the law may require disclosure of medical information for a variety of reasons unrelated to the actual provision of health care: as part of child custody cases, for educational services, for reimbursement of health care services, as part of U.S. Food and Drug Administration audits, and the like.[32]

## GENETIC TESTING

Discovery of a gene associated with increased risk for cancer is announced almost every week. For some, genetic tests are available to determine whether a person has a risky mutation. Discovery of these cancer predisposition genes has raised a multitude of difficult questions: Who should be tested and under what circumstances? How should the genetic information related to cancer predisposition be handled?

Genetic tests are perceived to be different from other diagnostic medical tests for several reasons.[34] First, genetic tests have implications for the health of other family members; a germ line mutation in one member means that other relatives could be affected. Second, because of incomplete penetrance, genetic tests usually entail information about risks and probabilities. This factor complicates deliberations about their implications for particular patients. Finally, although all genetic testing raises the issues of psychological risks, the effect may be particularly pronounced for children, because so many fundamental life choices—education, career, marriage, reproduction—are yet to be made.

In discussing genetic testing, it is important to be clear that the ethical considerations in performing a genetic test vary, depending on whether the test will be performed in a clinical setting or in a research context. In the clinical setting, genetic testing can be performed for either diagnostic testing or screening purposes among high- or average-risk individuals. Currently, a consensus maintains that in no situation should genetic testing be recommended for cancer screening purposes.[35,36] More controversy surrounds the clinical use of genetic testing for determining an individual's cancer predisposition. Most agree on the two broad criteria that should be used to determine the appropriate time to use genetic testing in clinical care.[35,37] First, actual test results must be reliable, valid, and interpretable (i.e., a test must allow for accurate performance, with sufficient knowledge about what a particular result means for the risk of developing cancer). Second, the result must have practical implications for health care [i.e., clinical genetic testing should be undertaken when it might result in (a) instituting an effective preventive intervention; (b) screening with the potential for early detection and effective treatment; or (c) altering reproductive choices]. Disagreement arises as to whether these criteria are satisfied in any cases related to cancer predisposition testing. Such criteria probably are met in multiple endocrine neoplasia type IIb, which allows for a reliable test for *RET* gene mutations, and thyroidectomy is an effective prophylactic treatment. However, in other cases of cancer predisposition genes, such as *p53* for Li-Fraumeni syndrome, the current state of knowledge probably does not fulfill such criteria.[38]

In offering genetic testing in the clinical setting, great emphasis has been placed on ensuring appropriate informed consent.[34,36,39] Because genetic testing is rarely of great urgency, the informed consent process can occur over several meetings that include introduction of information, discussion of the implications of the test results, and appropriate counseling. Currently unclear is what types of information, such as brochures, videos, or discussions, provide the best mechanism for disclosure. In considering genetic testing in children, some have argued that they cannot provide informed consent and, therefore, that testing should occur only in the presence of clear preventive, treatment, or reproductive choices that must be made before majority.[34] In cases that demonstrate no clearly established medical benefits, it is "advisable to defer testing until adulthood," at which time children can make their own medical decisions.[40]

In the research setting, genetic testing can be performed on tissue collected as part of a protocol or on stored biologic samples. When tissue is collected as part of a protocol, investigators must be sure that the subjects provide informed consent and clearly understand the potential harms that might result.[39] Table 48-4 provides a list of items that should be included in the informed consent documents of clinical research protocols that will include genetic testing. In conducting research on stored biologic samples, it is important to distinguish between samples that can be linked to specific (identifiable) patients and those that have been "anonymized" (i.e., patient identifiers have been irreversibly expunged).[41] Current regulations do not require informed consent for samples that have been anonymized. Indeed, although federal regulations technically exempt such research from examination by an institutional review board, it is becoming increasingly common to have such review.[41] More important, it is becoming increasingly common to include explicit consent for future genetic research as part of clinical research studies that collect and will store biologic samples.

| |
|---|
| The nature of the proposed study |
| Identification of the research team |
| Privacy guidelines for the study |
| Plans for archiving samples, DNA, or cell lines |
| Distribution of samples beyond the primary study, as described in the consent form |
| Commercial implications of the research |
| The possibility of revealing sensitive biologic information (i.e., nonpaternity) |
| Implications of study results (i.e., possibility of informational harm to vital interests such as insurability or employability) |

From Clayton EW, Steinberg KK, Khoury MJ, et al. Informed consent for genetic research on stored tissue samples [see comments]. JAMA 1995;274(22):1786–1792, with permission.

**TABLE 48-4. ISSUES THAT REQUIRE DISCLOSURE TO POTENTIAL SUBJECTS WHEN OBTAINING INFORMED CONSENT TO PARTICIPATION IN GENETIC STUDIES**

Research using genetic testing also must indicate whether results of the genetic tests will be disclosed to patients or their physicians (or both). This practice obviously will affect the study design, specifically regarding whether to perform the genetic tests on identifiable or anonymized samples; if patients are not going to be informed of the results, anonymized samples should be used if at all feasible. However, with identifiable samples, results with clear clinical relevance should be disclosed to subjects, whereas those results that have no clinical relevance should not be disclosed.[42] Whether ambiguous results or those of uncertain clinical import should be disclosed is debatable. Whenever disclosure does occur, appropriate genetic counseling should be provided before and after testing.[43] Indeed, research assessing the impact of disclosing genetic test results should be part of any clinical research protocol that discloses such results, so that insight into psychological and other outcomes can be obtained.

## ETHICAL CONCERNS IN THE INTENSIVE CARE UNIT

## Clarifying the Goals of Care

At the transition of oncology patients to the ICU, it is especially important to revisit the overall care plan and to clarify expectations. One problem commonly encountered in this environment is that, in the minds of both families and clinicians, the goals of care often are dichotomized into curative versus palliative concerns. In the ICU, changes in the intensity of care often are relatively abrupt and dramatic. For example, for an oncology patient just being admitted to the ICU, this transition often entails new physicians and nurses along with a relatively dramatic escalation in the level of medical therapies. At the other extreme, for a patient who has just undergone a long and complicated trial of therapy and is dying despite maximal support, the dying process may be marked by the withdrawal of life-sustaining treatments. However, treatments intended to relieve suffering are not an alternative, or an abrupt redirection, from those intended to cure illness or prolong life. Ideally, both are management priorities that have existed in the care plan prior to transfer to the ICU, and both should remain management priorities while patients remain in the ICU. However, data from the literature suggest that this essential tenet apparently is not what is actually practiced. Wolfe et al. [44] found that of 44 pediatric oncology patients who died in the hospital, 20 died in the ICU. These investigators reported that the parents of children who died in the ICU were more likely to report symptoms of suffering than were parents of children who died in settings with a lower concentration of physicians and nurses available to diagnose and treat discomfort at the end of life.

These findings highlight the importance of establishing clear goals of treatment when care is transferred to the ICU. First, preferably before admission to the ICU or as soon after admission as possible, physicians should explain that aggressive therapies designed to cure illness or extend life can and will coexist with a priority to relieve suffering. Second (and equally important), physicians should alert the family and clinical team to the anticipated duration of a trial of life-sustaining treatment. Predicting the response to intensive care treatment in an individual case usually is not possible. However, it is possible for experienced clinicians to provide a good estimate of the duration of time beyond which curative expectations will diminish significantly. The simple step of having an early family meeting to clarify these points will help to reduce conflict later in the clinical course engendered by diverse expectations or feelings of abandonment.

Careful attention must be given also to word choice in these conversations. Questions such as, “Would you like to continue with aggressive care for your child, or start comfort measures?” frame the issue as “one or the other” and, predictably, lead to one response from parents. Rather, such phrases as “ensuring your child's comfort remains our chief concern; let's discuss what else we can do to make these final days meaningful” convey to the family ongoing commitment and concern by clinicians.

## Decision Making about Life-Sustaining Treatments

Dramatic advances in critical care medicine over the last 30 years also have prompted a realization that the temptation to use life-sustaining treatments because they are capable of maintaining biologic existence must be balanced with an understanding of when they are indicated and when they are not. A reflection of this concern has been the dramatic increase in the last decade of decisions to withhold or withdraw life-sustaining treatment in critically ill patients. All recent observational studies of patients who die in adult,[45] pediatric,[46] or neonatal[47] ICUs reveal that the majority dies after the decision to withhold or withdraw life-sustaining treatments. How clinicians should reason about end-of-life decisions also has received more scrutiny over this time. Recommendations for ethical decision making regarding limitations of life-sustaining treatments and for the appropriate care of these patients once a decision has been made were put forth as early as 1983 by the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. [48] Consensus guidelines and recommendations for clinicians on decision making for critically ill patients have been put forth also by the American Academy of Pediatrics and other professional organizations. [49,50,51,52,53,54 and 55]

Despite these consensus guidelines and recommendations, accumulating data in the literature document wide variation in the manner in which clinicians make decisions about life-sustaining treatments.[56] These studies suggest that variables independent of patients' preferences, such as physicians' attitudes and practice specialty, are better predictors of end-of-life decision making. [57] How should clinicians reason about the decision to withhold or withdraw life-sustaining treatments?

### *“Baby Doe” Regulations*

Perhaps the most controversial and misunderstood framework for decision making about life-sustaining treatments in pediatrics concerns the so-called Baby Doe regulations.[58,59] Born in Bloomington, Indiana, in 1982, Baby Doe was an infant with Down syndrome and tracheoesophageal fistula. His parents declined corrective surgery on the grounds that he would never achieve a “minimally acceptable quality of life,” and the child subsequently died. The case generated public controversy, with the subsequent promulgation of Department of Health and Human Services regulations designed to prevent parents or health care providers from withholding care from handicapped infants. After a number of appeals, the final Baby Doe regulation, often termed the *Final Rule*, was passed by Congress as the 1984 Amendments to the Child Abuse Prevention and Treatment Act.[59a,59b] This legislation required all states to create a regulatory system to investigate cases in which medically indicated treatment is withheld from handicapped infants; failure to do so would subject them to risk of withholding of federal funding for children's services. The legislation also stipulated that “the withholding of medically indicated treatment from a disabled infant with a life-threatening condition” by parents or providers was considered medical neglect. The legislation further outlined three medical conditions that would justify withholding of otherwise required treatment. According to stipulations in the final rule legislation:

> “The term “withholding of medically indicated treatment” means the failure to respond to the infant's life threatening conditions by providing treatment (including appropriate nutrition, hydration, and medication) which, in the treating physician's reasonable medical judgment, will be most likely to be effective in ameliorating or correcting all such conditions, except that the term does not include the failure to provide treatment (other than appropriate nutrition, hydration, or medication) to an infant when, in the treating physician's reasonable medical judgment any of the following circumstances apply:”
>
> I. The infant is chronically and irreversibly comatose;
> II. The provision of such treatment would merely prolong dying, not be effective in ameliorating or correcting all of the infant's life-threatening conditions, or otherwise be futile in terms of survival of the infant; or
> III. The provision of such treatment would be virtually futile in terms of survival of the infant and the treatment itself under such circumstances would be inhumane.

Many argue that the Baby Doe regulations are not helpful in decision making for infants because of ambiguity surrounding the term *appropriate*. Regardless of how one interprets the intentions of the final rule legislation, this framework is not recommended commonly for ethical decision making at the end of life in the pediatric patient. Rather, it is viewed as a regulatory statute without common clinical application. [60]

### *Futility and End-of-Life Care*

Another controversial concept encountered in end-of-life decision making is the notion of futility. Conflicts arise between physicians and parents when agreement cannot be reached over whether life-sustaining therapies should be initiated or continued. Typically, such disagreements can be resolved through ongoing discussions. Indeed, what at first appears to be a conflict about whether a treatment is futile may be more related to a grief reaction associated with an affected child's dying. In such cases, what is required is not confrontation but careful listening, empathetic support, and continued discussions.

In some situations, however, dialogue leads not to agreement but to conflict between parents and physicians over whether a life-sustaining treatment is futile. The most publicized pediatric case in this regard concerned Baby K. [61] The infant Baby K was born with anencephaly in Virginia in the early 1990s. Baby K's mother demanded that physicians provide mechanical ventilation for her daughter during multiple hospitalizations for aspiration pneumonia. The clinicians refused, stating that mechanical ventilation could not reverse the anencephalic infant's malformation and therefore was not indicated. Her mother responded by saying that she understood the medical facts about anencephaly and the natural history of the malformation “but the value of this kind of life is God's secret.” That is, there was no disagreement about the facts of the case, but these “medical facts” were valued differently. In such a situation, whose values predominate? [61] Can patients or their surrogates demand care that clinicians believe is against their professional conscience? In the case of Baby K, a federal appeals court ruled that the hospital was required to provide care to any patient seeking emergency treatment as dictated by the Emergency Medical Treatment and Active Labor Act. [62]

Defining situations of true futility, however, is difficult in practice. One widely cited definition proposed that “when physicians conclude (either through personal experience, experiences shared with colleagues, or consideration of published empiric data) that in the last 100 cases a medical treatment has been useless, they should regard that treatment as futile. If a treatment merely preserves permanent unconsciousness or cannot end dependence on intensive medical care, the treatment should be considered futile.” [63] Yet, this and other attempts to define *futility* now are seen as inherently flawed because any predetermined thresholds (such as 100 cases) are ultimately arbitrary, and each patient's situation is unique. [64] Not only are patient circumstances different, but physicians do not universally agree

about how to define *futility*. Finally, people may differ over the value of a treatment, as in the case of Baby K. For instance, some may value preserving life at all costs, whereas others may conclude that the quality of life is so poor that death is the preferred outcome. Further, some may see hope in extremely small odds for success ("still hoping for a miracle"), whereas others see a prolongation of the dying process. [65,66] Who is right?

The Society of Critical Care Medicine states that treatments should be defined as futile only when they will "not accomplish their intended goal." [67] Moreover, thisofficial position regarding futility by the largest professional body of practitioners of critical care medicine states [67]:

> "Treatments that are extremely unlikely to be beneficial, are extremely costly, or are of uncertain benefit may be considered inappropriate and hence inadvisable, but should not be labeled futile. Futile treatments constitute a small fraction of medical care. Thus, employing the concept of futile care in decision making will not primarily contribute to a reduction in resource use. Nonetheless, communities have a legitimate interest in allocating medical resources by limiting inadvisable treatments."

This approach advocates that local communities draft procedures to be followed in cases of dispute, with broad input from the community, instead of *ad hoc* attempts at the bedside to define and resolve differences over the meaning of futility. The Society of Critical Care Medicine policy goes on to state that

> "[c]ommunities should seek to do so using a rationale that is explicit, equitable, and democratic; that does not disadvantage the disabled, poor, or uninsured; and that recognizes the diversity of individual values and goals. Policies to limit inadvisable treatment should have the following characteristics: (a) be disclosed in the public record; (b) reflect moral values acceptable to the community; (c) not be based exclusively on prognostic scoring systems; (d) articulate appellate mechanisms; and (e) be recognized by the courts."[67]

In summary, despite an ethical and legal consensus that physicians have a duty to discontinue therapies that have been refused by patients, it does not extend to a parallel duty to fulfill all requests that have been made by patients.

### *Guideline for Decision Making*

If the Baby Doe regulations and futility guidelines are not helpful for bedside decision making about life-sustaining treatments, do physicians have any accepted guides? The framework put forward by a president's commission in its 1983 publication, *Deciding to Forgo Life-Extending Treatment*, frequently is cited as a useful construct.[48] The president's commission proposed five considerations for determining a child's best interests in these situations:

1. The amount of suffering and the potential for relief
2. The severity of dysfunction and the potential for restoration of function
3. The expected duration of life
4. The potential for personal satisfaction and enjoyment of life
5. The possibility of developing a capacity for self-determination

The commission then advocated applying these criteria to proposed treatments for children on the basis of three assessments of the proposed treatment plan: clearly beneficial; ambiguous or uncertain; and futile. The commission further stated that if the proposed treatment is either ambiguous or uncertain or futile and the parents prefer to forgo treatment, clinicians should withdraw life-sustaining therapy.

### Moral Accountability

Some clinicians believe that such distinctions as "ordinary versus extraordinary" or "withholding versus withdrawing" are helpful in guiding practice as they reason about ethical considerations in end-of-life decision making. The presumption is that providing treatments that are classified as ordinary is mandatory, whereas forgoing treatments that are extraordinary is morally permissible. Similarly, some claim that withholding treatments is morally permissible but withdrawing them is not. Although this terminology is used commonly by some, most ethicists believe that these distinctions confuse rather than clarify actions at the end of life. [68]

Consider the ordinary-extraordinary distinction. One interpretation would be that ordinary treatments are obligatory, whereas extraordinary treatments are not. A simple appeal to what is customary, however, cannot suffice as a justification for what is morally required. The argument is essentially circular, as it claims that ordinary treatments are morally required because they are ordinary and extraordinary treatments are morally optional because they are extraordinary. Whether something is ordinary or frequent does not determine whether it is morally required.

Still others cite a distinction between withholding and withdrawing treatments. Is there a moral difference between deciding not to intubate a patient, because physicians do not think that the patient will benefit from mechanical ventilation, and extubating a patient who has failed to respond despite a period of mechanical ventilation? In the landmark Nancy Cruzan case, the U.S. Supreme Court stated that no legal difference exists between the two actions. [69,70] In addition, abroad ethical consensus maintains that such a distinction is not morally relevant. Understandably, one feels more responsible for the outcome when it results from the withdrawal of a therapy than for the outcome that results from withholding of a therapy. Part of the reason is clearly psychological and should not be dismissed lightly. Nevertheless, no fundamental logical difference applies between the two decisions to render one more morally acceptable than the other. Indeed, many ethical experts have stated that it may be preferable to favor withdrawal after an initial trial of treatment, especially in the presence of doubt about the decision whether to start a therapy. Initiating a trial of therapy in situations of prognostic uncertainty allows for a chance to determine whether the benefits outweigh the burdens. [53,71,72]

### Sedatives and Analgesics in the Care of the Dying

Despite attempts to educate and encourage the medical community regarding pain control in recent years, only limited progress has been made. In the SUPPORT Investigation, a study that examined the dying process of more than 9,000 seriously ill adult patients in American hospitals, surrogates reported that 50% of patients who could communicate reported moderate or severe pain at least one-half of the time during their last 3 days of life. [73] More recently,Wolfe et al.[44] found that 89% of 103 parents whose children died of cancer in a hospital setting reported that their children experienced suffering "a lot" or "a great deal" from at least one symptom in their last month of life. In the children who were treated for specific symptoms, treatment was successful—by the parents' assessment—in only 27% of those with pain and in only 16% of those with dyspnea. These data suggest the need for more effective management of patient suffering and clearer communication with parents in the care of children who are dying of cancer.

No prohibition in bioethics or religious traditions prevents physicians from treating the pain and suffering of terminally ill patients, even when such treatment may hasten an affected child's death. Similarly, the U.S. Supreme Court has supported this concept. [74] The ethicalprinciple relevant to this question is the doctrine of double effect, originally developed by Catholic theologians but subsequently accepted broadly by other religious traditions and by law and philosophy. [75] The doctrine states that when an action has two effects, one of which is inherently good and the other of which is inherently bad, it can be justified if certain conditions are met.

The doctrine of double effect has as its parameters four requirements. First, the action in itself must be good or at least morally indifferent. Second, the agent must intend only the good effect and not the evil effect; the evil effect is foreseen, not intended; it is allowed, not sought. (In the case of administering morphine to a terminally ill patient, the physician must intend only the relief of the patient's pain and suffering. Respiratory depression and the potential for an earlier death is a foreseen complication but is not sought.) Third, the evil effect cannot be a means to the good effect. [If the physician administers a bolus of potassium chloride instead of morphine, this condition would be violated. By administering potassium chloride, the evil effect (death) becomes the means to the good effect (relief from suffering). By contrast, morphine does not depend on the side effect of death to relieve pain effectively.] Fourth, the good intended must outweigh the evil permitted. (In the case of an imminently dying patient, the benefit of pain relief clearly outweighs the risk of death. This would not be true if the patient were not terminally ill.) For example, if an otherwise healthy patient required so much morphine for pain control that serious respiratory depression developed, that patient should be placed on a ventilator and not allowed to die.

Whether double-effect reasoning is a necessary or sufficient guide for morally permissible administration of sedation and analgesia in terminal care is controversial. Critics of the doctrine of double effect argue that it depends on an overly simplistic notion of intent that is impossible to verify externally. These critics believe that the only morally relevant consideration is the informed consent of the patient, not the intentions of the clinicians. [76] Others claim that strict adherence to the doctrine of double effect may have the paradoxical effect of constraining some clinicians from providing adequate medication for relief of suffering because of their fear of violating the absolute prohibition against intentionally causing death. In the ICU, however, no viable alternatives to double-effect reasoning aid in guiding permissible

clinical conduct. Most terminally ill children in the ICU cannot express their wishes for terminal care, and most will experience a rapid decline in comfort as life support (e.g., mechanical ventilation) is withdrawn. Double-effect reasoning provides a defensible rationale for escalating doses among practitioners who support neither euthanasia at one extreme nor allowing patients to die with untreated suffering at the other.

What is the difference between practice by the doctrine of double effect and the performance of euthanasia? The key difference lies in the intention of physicians. Although the full intentions and motives of another cannot be validated with certainty, if a physician's intention is treatment of a patient's pain and suffering, the administration of analgesics and sedatives is ethically permitted under the doctrine of double effect. When a physician's intention is to kill a patient, the line between accepted practice and euthanasia has been crossed.

How much analgesia or sedation is too much for a terminally ill child? No arbitrary amount of narcotic is necessarily too much, or too little, in any given case. Brody et al.[77] have expressed the views of many experts on pain and symptom management: “The optimal dose of morphine for relief of pain or dyspnea is determined by increasing the dose until the patient responds. Patients who have not previously received opioids should initially be given low doses, which should be rapidly increased until symptoms are relieved. For patients with particularly severe or acute symptoms, rapid titration requires that an experienced clinician be at the bedside.” One of the few pediatric studies to examine the amount of narcotic administered to dying patients found that 94% of 199 children who died of malignancy were managed with morphine equivalent doses of less than 3 mg per kg per hour as part of their terminal care. The need for doses greater than 3 mg per kg per hour in 11 of 12 patients was associated with the diagnosis of metastases to the central nervous system.[78] Regardless of the dosing scheme required to treat pain and suffering effectively, standard medical practice should be to document thoroughly the signs and symptoms of suffering that the clinicians observe and the rationale behind the regimen chosen to treat these symptoms.

Another issue peculiar to end-of-life care in the ICU concerns the use of neuromuscular blocking agents in children with severe lung disease requiring mechanical ventilation.[79] These agents never should be introduced as mechanical ventilation is being withdrawn from a patient, because they have no analgesic or sedative properties and may mask symptoms of suffering. Similarly, in patients who already are receiving neuromuscular blocking agents, neuromuscular function should be restored before the life support is withdrawn. In limited circumstances, possibly the burdens to patient and family of waiting for the neuromuscular blockade to diminish to a reversible level exceed the benefits of allowing better assessment of a patient's comfort. In these cases, many bioethicists have stated that it may be morally permissible to proceed with the withdrawal of mechanical ventilation after an open discussion between the family and clinicians of the benefits and burdens of alternative courses of action.[77,79]

### Withholding Medical Nutrition and Hydration

A consensus in bioethics holds that the withdrawal of medical nutrition and hydration should be justified by a burdens-benefits assessment of that therapy for a patient, just as it is for decisions to withdraw mechanical ventilation and all other forms of life-sustaining treatment.[80] An important note is that, in this context, use of the terms *medical nutrition* and *hydration* assumes that affected patients are dependent on enteral or parenteral nutrition and have no suck-and-swallow capability to sustain themselves by mouth feeding. For many, however, to withhold something so basic to human existence—even for patients dependent on enteral or parenteral nutrition because of a persistent vegetative state—is to diminish the dignity of such patients and the humanity of the caregivers. Similarly, because of the symbolic significance of providing nutrition and hydration, which to many is the very core of nurturing care, forgoing their use might seem to be the abandonment of a patient. Despite these concerns, courts have ruled that medically administered nutrition and hydration should be considered medical interventions like any other and may be discontinued on the same grounds as any other medical treatment. This position posits the absence of a logically valid distinction between the withholding or withdrawal of a tube from the trachea providing life-sustaining treatment and the withholding or withdrawal of a tube from the intestine providing life-sustaining treatment. In the Cruzan case,[81] the U.S. Supreme Court ruled that artificial hydration and nutrition are considered medical therapies, which patients or their surrogates speaking for them have a constitutional right to refuse. Concurring with the majority ruling, Justice Sandra Day O'Connor concluded, “Artificial feeding cannot readily be distinguished from other forms of medical treatment . . . Accordingly, the liberty guaranteed by the Due Process Clause must protect, if it protects anything, an individual's deeply personal decision to reject medical treatment, including the artificial delivery of food and water.”[81]

### Ethical Considerations after Death

After the death of a patient, additional ethical concerns are encountered. Autopsies often are an important source of information in pediatric oncology for both the involved physicians and the families of children who have died. Despite the use of highly sophisticated noninvasive diagnostic technologies, studies continue to show that autopsies frequently reveal important diagnoses that were not suspected before death.[82] Pediatric oncologists, therefore, need to be competent in the process of seeking consent for autopsy from families. However, one recent study[83] found that 51% of 200 chief residents in internal medicine and pediatrics at U.S. teaching hospitals reported substantial deficiencies in their knowledge of the autopsy procedure, including the fact that organs are not routinely returned to the body for burial.

The amount of information about autopsy procedure necessary to persuade a family to give full, informed consent to a limited autopsy is not intuitively obvious, and providing details about the autopsy procedure may itself create serious distress. Affected physicians also may not be aware of the religious or cultural beliefs of a patient's family, beliefs that often are heightened at times of acute illness and death and may be the source of the values that the family will apply to decisions about whether to consent to autopsy. Rather than seeking to master the nuances of multiple cultures, it may be more practical to ask open-ended questions, such as, “What are the most important results you would hope to receive from an autopsy? What are the most important concerns that you have about autopsy?”

Physicians should ensure also that requesting physicians have “earned the right” to request an autopsy by virtue of their involvement in the care of affected patients and their relationship with such patients' families. Finally, language that is used should explain clearly the general procedures that will be followed (e.g., “We request your permission to donate your son's organs so that we may better understand why the cancer spread. This information will be of potential help not only to you but to future families”). In addition, families of deceased patients should understand also that they have the right to request restrictions to the autopsy but that limitations will increase the likelihood of incomplete information.

## FINANCIAL INCENTIVES AND CONFLICT OF INTEREST

### Definitions

A conflict of interest is said to exist when a set of conditions in which professional judgment considering a primary interest is or may be influenced unduly by a secondary interest. Physicians' *primary interest* is the health and welfare of their patients. Other primary interests for physicians in academic settings are the integrity of research or the education of the next generation of physicians. *Secondary interests* may be important parts of professional practice and may provide physicians with access to the resources necessary to offer benefit to patients.[84] The discussion here focuses on financial conflicts of interest, which are easiest to identify and regulate, including financial benefit accruing to a physician or to an institution in which the physician is practicing (private or group practice, hospital, or health maintenance organization).[85]

### Types of Conflicts of Interest

As medicine has become much more than a business and has emerged as a huge industry, conflicts of interest have become more complex. No longer are direct financial incentives to a physician the major source of conflicts of interest affecting patient care and options. The policies and reward systems inevitably affect physicians practicing as employees of large corporations. Areas of special concern are physician referrals to entities that they own or in which they have a financial interest, gifts from drug companies, and industry-sponsored research. Incentives affect clinical practice, usually with the goal of maximizing revenue or controlling costs. Additional issues arise if physicians managing patients are also clinical investigators and if institutions established to bring the benefits of new knowledge to the bedside are dependent on industrial contacts and support for access and resources.[86] The complexity of these factors in oncology—and pediatric oncology in particular—mandates special attention to recognition, disclosure, and management of conflicts of interest. Some conflicts are inevitable and may arise from desirable overlaps that enhance patient access to limited treatment resources, including new drugs.

### Professional Behavior

All therapeutic relationships are based on *trust.* Actions that undermine the trust between physicians and patients or patients' families or between such families and the institutions to which they entrust the lives of their children damages the basis of therapeutic benefit. Addressing conflicts of interest so that such damage is

avoided is a professional responsibility shared by physicians, hospitals, medical centers, and research entities.

The same factors that drive all humans (altruism, recognition, advancement, material rewards, and a desire to make a difference) motivate physicians. Most pediatric oncologists practice in the setting of large health care institutions, and many are in academic positions. The rewards, incentives, and disincentives offered by those institutions exert pressures on pediatric oncologists, surgeons, irradiation oncologists, nurses, and others involved in the care of children with cancer. Even when these pressures do not affect physicians directly, the perception of whether a third party is likely to pay may affect the options offered or the therapies advocated. Some of the motivators that may affect the practice of physicians are listed in Table 48-5.

| |
|---|
| Altruism |
| Personal emotional investment in relationship with a patient or family |
| Professional advancement |
| Promotion and tenure |
| Professional recognition and reputation |
| Sense of personal accomplishment |
| Advancement within a group (local, regional, national) |
| Avoidance of responsibilities that are not rewarded (not reimbursed financially, unwanted phone calls or patient encounters) |
| Advancement of institution |
| Building referrals or a program important to local institution |
| Building programs in regional or national group, such as cooperative group |
| Control of costs by withholding therapies |
| Direct financial gain to physician—personal financial incentives (more or less money for conducting or ordering tests or therapies, self-referral, industrial incentives for enrollment on sponsored study) |
| Indirect financial gain to physician: grant funding, support for activities important to physician |

**TABLE 48-5. INTERESTS THAT MAY AFFECT PRACTICE PATTERNS OF PHYSICIANS**

Less tangible factors, particularly those that promote a reputation or sense of accomplishment, may be very powerful motivators. Enhancement of professional reputation in an academic setting may be much more important to a faculty member than is the financial incentive of promotion or tenure. This is much harder to identify, quantitate, and regulate than are financial incentives. Vigilance, self-awareness of physicians, and education and sensitivity of institutional leaders are important in management of nonfinancial incentives.

Physicians often feel that they are being accused of unethical behavior when the issue of conflict of interest is raised. Recognition and appropriate management are important to maintenance of public trust and professional integrity. Several generally applicable concepts may be useful to approach the topic. [87]

### Conflicts of Interest in Medicine and Science

Physicians who earn a living by recommending, prescribing, and dispensing therapies and interventions to patients inevitably create and encounter situations in which they may benefit. In a fee-for-service system, it is financially advantageous to physicians to recommend more therapy or more tests. Billing for daily inpatient visits provides an incentive to have patients complete a course of chemotherapy or antibiotics as inpatients rather than at home. Physician-entrepreneurs could benefit from investing in and owning facilities to which they refer their own patients. This possibility has precipitated regulation of these relationships because of the difficulty in determining which interests are paramount.

Practice in an institutional setting renders physicians subject to pressures to conform to institutional norms and policies. The fiscal health of any health care institution is essential to its survival. Policies that potentially are not in the best interest of patients may be in place to advance or protect an institution. Physicians must advocate for their patients while recognizing economic realities, often creating tension. Recognition of the validity of the claims of all parties is key to management of such conflicts.

Similar issues may arise in clinical research. An exciting new agent or test attracts the interest of many investigators. An agent or diagnostic modality arouses the interest of investigators who perceive the new approach as promising. The enthusiasm that leads an investigator to commit to enrolling patients on a study may affect judgment when it comes to assessing results and interpreting data, even without the personal financial incentive of ownership or investment.

### Varying Impact of Secondary Interests

Not all secondary interests are equally likely to affect professional judgment. In general, the larger the secondary interest, the more likely it is to affect professional judgment. Below a certain level—which varies with individuals and with circumstances—an incentive, such as a gift from a drug company, may exert little or no effect. Relationships that are closer and more long-standing are likely to have an impact greater than that of a one-time interaction. The independent authority of physicians creates an opportunity for a secondary interest to exert more impact than that of the same interest for professionals with less discretion in their actions.

Individual physicians and groups may benefit from their own investments or ownership of resources providing care. Rewards for providing tests or therapies may be considerable.[88] Practicing physicians who are also laboratory directors may derive considerable portions of their income from tests that, by virtue of their positions, they can order or encourage their colleagues to order. Investment in a pharmaceutical company offers an incentive to prescribe agents produced by that company.

State-of-the-art care of complex patients requires access to diagnostic procedures and therapies available in large academic institutions or smaller institutions with community support. The incentives can be strong to use those clinical services that generate excess revenue. In capitated systems, withholding specialized care to control costs may be economically advantageous. A self-insured group, such as a small business, can be devastated by a catastrophic illness in one of its members. In this instance, the incentives can be strong to withhold care.

Development of new pharmaceutical agents and diagnostic techniques usually is supported by industry. [86] Decisions about where to invest resources are economically driven: Not only which agent is most likely to be effective but what the potential market may be determines lines of investigation. Pediatric oncology has been relatively protected from the most intense economic pressures in this field by the fact that the market is so small. Recent federal incentives to develop drugs for treating children may increase the economic incentives to pediatric oncologists to engage in drug development for profit. The very considerable economic benefit, particularly in the context of lower reimbursement for other activities, creates very powerful conflicts for physicians.

### Patient Benefits and Risks from Secondary Interests

Secondary interests are not intrinsically good or bad. Physicians enhance their ability to benefit their patients by having access to knowledge, resources, drugs, and technology. Whether these resources advance the welfare of their patients depends on the circumstances. The importance of these interests is affected by the magnitude of the damage that may be inflicted on patients (or on the integrity of clinical research, in an investigative context) and by the magnitude of the conflict.

Physicians who are able to make decisions with little accountability to disinterested parties must exert particular care to avoid conflicts of interest. Decisions by physicians or investigators who are able to offer unique resources by virtue of their secondary relationships and interests must be subject to review by colleagues who do not have such conflicts. Some case examples of conflicts of interest are detailed in Table 48-6.

**TABLE 48-6. SOME CLINICAL EXAMPLES OF CONFLICT-OF-INTEREST SITUATIONS**

### Common Behavioral Guidelines to Prevent Harm

The practice of pediatric oncology and the performance of clinical research that advances the care of children are complex, multi-institutional enterprises governed by rules and regulations. The fundamental basis of all protections is *integrity*: the integrity of physicians caring for patients and of investigators performing research. The medical profession has a disappointing track record of self-regulation, with the result that governmental regulation is increasing. Rules affecting conflict of interest are designed to protect the integrity of and public confidence in professional judgment.

Removal of all secondary interests is not necessary to minimize their influence on decisions about primary interests. The actions that must be taken vary, depending on the magnitude of the secondary interests and the stakes in the primary decision.

It is important to maintain the appearance of integrity so that reasonable people would be unlikely to conclude that professional judgment has been unduly influenced. Patients' trust in physicians and the public's trust in the profession are at risk. In pediatric oncology, the reputation of and public trust in clinical research also are at stake because of the integration of clinical research with standards of clinical care. [89]

### Management of Conflicts of Interest

The measures to be taken to manage conflicts of interest vary, depending on several factors:

- Financial magnitude of the secondary interest
- Importance of the secondary interest to affected physicians or investigators, with close and long-standing relationships relatively more important than transient or arm's-length relationships
- Degree of physicians' or investigators' accountability in exercising judgment and review of decisions. If there is no review by fully disinterested parties, it is more important to avoid secondary interest that could influence decisions
- Amount of damage that can be inflicted if conflicts exert undue influence (including harm to the profession or scientific endeavors as a whole)

### Options for Dealing with Conflicts of Interest

The first level of dealing with conflicts of interest is *disclosure.* At this level, physicians or investigators must disclose that a secondary interest exists. Physicians must tell patients of their financial interest in a laboratory to which they are referring them, and investigators must disclose the source of support for research. Disclosure, however, is a very limited remedy, because it reveals a problem without providing any approach to a solution: The interpretation and response to disclosure are left up in the air. Most patients and colleagues have no idea about how to respond to a disclosed secondary interest. This is an area in which considerable work is needed.

A second level of response to a potential conflict is to introduce an intermediate, or *mediator.* This is the purpose of a blind trust, commonly created when private citizens enter politics or assume a governmental position. It is designed to limit the impact of a secondary interest while not totally eliminating it.

Physicians and researchers also may withdraw from discussions and decisions in which they have a secondary interest. This process, termed *abstention*, does not eliminate the conflict but also minimizes the impact of the secondary interest by removing the conflict from this particular decision. It is close to impossible to continue to be a managing physician without having input into significant therapeutic decisions regarding affected patients; thus, this approach is better for researchers than for physicians acting primarily as clinicians.

Finally, *divesting* oneself of the secondary interest eliminates the conflict. This step may be necessary if it is not possible to create situations in which the integrity of the professional relationship is maintained in the face of a secondary interest.

In summary, conflicts of interest are an increasing issue in medical practice and in clinical research. They are likely to continue to increase, given the overlap of patient care and research, the involvement of industry to provide patients with access to new technology, the powerful and conflicting pressures of the health care industry, and incentives to offer or withhold therapies in response to the economic interests of third parties. Such conflicts cannot be eliminated but only managed. The primary interest of physicians is the health and welfare of their patients. This fundamental precept directs the management of all secondary interests.

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# LATE EFFECTS OF CHILDHOOD CANCER AND ITS TREATMENT

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## INTRODUCTION

Of the approximately 7,500 U.S. children younger than age 15 found to have cancer each year, some 80%, or nearly 6,000, can expect to be cured of their disease. [1] The prevalence of U.S. childhood cancer survivors among young adults (ages 15 to 45), currently estimated at 1 in 900 persons, is expected to increase to as many as 1 in 250 persons in the year 2010.[1] Among just the U.S. college-age population (ages 18 to 21), approximately 67,000 are childhood cancer survivors (WA Bleyer and JJ Lee, *unpublished data*). Regardless of whether these astonishing estimates are accurate, they do reflect the undisputed progress made in pediatric oncology over the last few decades. They also underscore the need to screen survivors of childhood cancer for late effects of cancer therapy, because almost one-half these survivors are likely to have or to develop disabilities that alter quality of life. [2]

Several generalizations can be made about the types of late effects that may be anticipated on the basis of the specific therapy to which patients were exposed and the age at the time of that exposure. For example, with radiotherapy, adverse effects usually are not apparent immediately and are more likely to surface after a latent period (see Chapter 13). Conversely, chemotherapy is most likely to result in acute toxicities that usually are transient but occasionally persist (see Chapter 10). Because most chemotherapeutic agents are cell cycle–dependent, their acute toxicities can be related to the proliferation kinetics of individual cell populations. Most susceptible are those tissues or organs with high cell turnover rates, such as the bone marrow, orointestinal mucosa, testes, epidermis, and liver. Least susceptible are those cells that either do not or replicate slowly (e.g., neurons, muscle cells, connective tissue). Exceptions to this correlation exist: vinca alkaloids, methotrexate, cisplatin, ifosfamide, and high-dose cytosine arabinoside may cause neural damage; methotrexate and corticosteroids may injure bone; and anthracyclines may harm the heart. Injury that does occur to tissues with low repair potential tends to result in a long-lasting or permanent deficit. Thus, although children tolerate the acute toxicities of therapy better than do adults, growing children may be more vulnerable to the delayed adverse sequelae of cancer therapy, such as effects on growth, fertility, the myocardium, and neuropsychological function. Also, because children tolerate greater doses or dose intensities of most chemotherapeutic agents as compared to those in adults, they often receive more chemotherapy and surgery. In this situation, the higher threshold for acute toxicities during childhood results in a higher rate of delayed or chronic toxicities that become apparent years after the exposure. Examples are myocardial dysfunction after anthracycline exposure, osteonecrosis after corticoid therapy, hypertension after nephrectomy, hypersplenism after hepatic irradiation and hepatotoxic chemotherapy, and thyroid neoplasms after neck irradiation.

This chapter reviews many of the late effects seen in survivors of cancer and the manner in which these effects relate both to individual therapeutic modalities (surgery, irradiation, or single- and multiple-agent chemotherapy) and to combined-modality regimens, including those used for bone marrow transplantation (BMT; Table 49-1). The psychosocial and financial aspects are reviewed in Chapter 46 and Chapter 47. Differences in susceptibility between pediatric and adult patients are emphasized, to indicate situations in which recognition of late effects already has provided a rationale for modifying therapy, and to suggest reasonable starting points for evaluation of specific long-term problems (Table 49-2 and Table 49-3).

**TABLE 49-1. LATE EFFECTS OF ANTICANCER THERAPY**

**TABLE 49-2. SUGGESTED EVALUATION FOR SUSPECTED LATE EFFECTS**

| |
|---|
| History of any intercurrent illnesses |
| Review of systems |
| Development of any benign tumors or other cancers |
| Medications: prophylactic antibiotics |
| Educational status |
| Grade completed; special education classes? |
| Grade point average; results of intelligence quotient tests |
| Areas of weakness |
| Employment status |
| Insurance coverage |
| Individual policy, or coverage through parents? |
| Difficulties in obtaining insurance? |
| Marital history |
| Menses, libido, sexual activity |
| Pregnancy outcome (patient or spouse) |

**TABLE 49-3. INFORMATION TO OBTAIN DURING FOLLOW-UP CLINIC VISITS FROM SURVIVORS OF CHILDHOOD CANCER**

## GROWTH

Decreased linear growth is a common problem during therapy in children with cancer. Although catch-up growth may occur, such that the premorbid growth status is regained, in some instances short stature is permanent or even progressive. For example, severe growth retardation, defined as a standing height below the fifth percentile, has been observed in as many as 30% to 35% of survivors of childhood brain tumors [3,4] and [5] and in 10% to 15% of patients treated with some antileukemia regimens.[6,7] Although growth in patients with tumors involving the head and neck, other than the brain, has not been studied exhaustively, some reports indicate severe growth retardation in these patients as well. [2,8,9,10,11,12] and [13]

Comparison of the growth curves of children with acute lymphocytic leukemia (ALL) treated with different forms of central nervous system (CNS) prophylaxis has identified whole-brain irradiation as the principal cause of short stature. [6,14] The effects of cranial irradiation appear to be age- and dose-related. Children younger than 5 years at the time of therapy are particularly susceptible to its growth-inhibiting properties. [15] With conventional fractionation schedules, doses in excess of 3,000 cGy to the hypothalamus or pituitary gland result in severe growth retardation in more than 50% of patients with brain tumors [4,5] (Fig.49-1). In contrast, long-term growth retardation after 1,800 or 2,400 cGy, as used in children with ALL, is less frequent, generally is milder, and usually is not a problem. [16,17] and [18]

**FIGURE 49-1.** A 17-year-old boy with medulloblastoma was treated with 5,400 cGy to the posterior fossa and 3,600 cGy to the rest of the neuraxis. The patient's height before treatment was at the twenty-fifth percentile; 10 years after irradiation, he is well below the fifth percentile; sitting height also is less than the fifth percentile. Parents and siblings are all at least in the twenty-fifth percentile.

Conversely, although few children with ALL fall below the fifth percentile for height, large numbers of children have remained above the fifth percentile but nevertheless have experienced significant decreases in height percentiles from the time of diagnosis. In two large series of patients whose only irradiation had been 2,400 cGy to the cranium, an excess in the proportion of patients in the lower percentiles was noted as compared with that expected in a healthy population. [7,15] Similar changes in the distribution of height percentiles have been observed after 1,800 cGy cranial irradiation, [7,14,19] but whether the reduction in ultimate stature is less than that seen with 2,400 cGy is not clear. As long-term survivors of childhood ALL actually reach adulthood and their final heights can be measured rather than projected, the degree of loss of final height appears to be greater than previously was thought. [20,21]

The precise mechanism by which cranial irradiation induces short stature is not clear. Doses as low as 2,400 cGy have caused growth hormone deficiency, as indicated by uniform,[22,23] although sometimes transient,[24] blunting of spontaneous growth hormone pulses. However, other biochemical evidence of growth hormone deficiency, including low plasma insulin-like growth factor-1 (somatomedin C) levels and blunted growth hormone responses to different provocative stimuli, have varied.[5,16,25,26] Such abnormalities have not correlated fully with the degree of growth failure. Early onset of puberty in girls with ALL may contribute to loss of final height.

Direct inhibition of vertebral growth by spinal irradiation also contributes to short stature. This change is seen most commonly in brain tumor patients whose entire spinal columns have received doses in excess of 3,500 cGy.[27] Moreover, among children who had ALL and, in addition to receiving 1,800 to 2,400 cGy of craniospinal irradiation, received abdominal irradiation (1,200 cGy), almost 30% had standing heights in less than the fifth percentile. [7] This result may have been due to irradiation of the gonads or thyroid, to scatter radiation to the femoral heads, or both. Mathematical models that have been developed allow the degree of stature loss to be predicted on the basis of radiation dose, radiation port, patient gender, and ideal adult height. [28,29] When lower doses (1,000 to 2,500 cGy) have been given to ports, including part or all of the spine, patients, although not necessarily short, have reduced sitting heights (measured from crown to rump). [5,27,28,30,31] Such is the case for as many as 40% of long-term survivors of Wilms' tumor and Hodgkin's disease and may be more common with the higher radiation doses used for medulloblastoma and soft tissue sarcoma. This problem has been seen particularly in children who either are younger than 6 years or are undergoing their adolescent growth spurt at the time of radiotherapy.

Several excellent reviews detail the accruing experience with the long-term effects on growth of total-body irradiation (TBI). [32,33] and [34] After 1,000 cGy given as a single fraction, long-term severe decreases in growth rates appear in most children. Fractionation of TBI appears to decrease growth retardation. Thoracoabdominal preparatory radiation that occurs with total lymphoid irradiation also has been associated with short stature. [34] The pathogenesis of short stature in affected children often includes factors other than radiation, notably graft-versus-host disease (GVHD) and its therapy.

In contrast to the effect of radiation, growth retardation after chemotherapy alone usually is temporary, and patients usually catch up with their peers. Some chemotherapeutic agents, such as methotrexate and high-dose corticosteroids, appear to mediate this effect by direct inhibition on bone growth (see the section Musculoskeletal and Related Tissues). Although the long-term effects of intensive chemotherapy without radiation are yet to be determined, short courses of myeloablative chemotherapy appear not to have significant effects on height. [34]

In some studies, changes in body weight are more prominent than changes in height of long-term survivors of childhood cancer. Long-term weight loss may result from intestinal malabsorption (see the section Gastrointestinal Function). At the other extreme, obesity as measured by weight or body mass index has been reported in small groups of children with ALL and brain tumors treated with conventional therapy or bone marrow transplantation. [19,21,34,35] This problem has its onset either during therapy or within the first year after discontinuation of therapy and may either progress or stabilize. The majority of patients have been treated with cranial irradiation or TBI; the contribution of specific chemotherapies, particularly corticosteroids, to the development of obesity is unclear. Hypothalamic obesity in this setting has been associated with excess insulin secretion, and treatment with octreotide, a somatostatin agonist, induces insulin secretion, decreases leptin levels, and lowers body weight.[36] Another study of posterior fossa tumor survivors who had been treated with irradiation reported a reduction in body mass index after

initiation of growth hormone for short stature.[37] An association between obesity and learning disabilities after radiotherapy has been noted,[2] although it is more likely that both sequelae are due to prior therapy (see the section Neuropsychological and Neurologic Function) than that either causes the other.

Monitoring long-term survivors for growth problems relies on the use of standardized curves familiar to pediatricians. Because single values for heights and weights are unreliable for children, frequent serial measurements to establish each child's pattern of growth are recommended. Preferably, both sitting and standing heights should be obtained, if possible with a stadiometer, available in most endocrinology offices. Measurements should be recorded before therapy, every 1 to 3 months during therapy for the first year thereafter, and then once or twice annually until linear growth is complete. Because of the foregoing concerns about obesity after the termination of antileukemia therapy, particular attention should be paid also to weight during that time, especially during the first year.

For children whose growth is abnormal, the additional workup shown in Table 49-2, usually performed with the help of appropriate consultants, may be undertaken. However, even in children in whom growth hormone deficiency has been documented before epiphyseal fusion, clinical responses to growth hormone sometimes have been suboptimal.[38,39] Moreover, an association, albeit highly tenuous, between exogenous growth hormone and the recurrence or development of leukemia should temper the use of this therapy in children with minimal loss of height.

Current approaches to cancer therapy in children include attempts to spare adverse effects on growth. Leukemia protocols are attempting to use intrathecal chemotherapy, high-dose methotrexate, high-dose cytosine arabinoside, or other CNS-active systemic chemotherapies in lieu of radiation for CNS prophylaxis (see Chapter 19). Hodgkin's disease protocols for smaller children have limited radiation doses successfully by the addition of chemotherapy (see Chapter 23). Hyperfractionation schedules for radiotherapy and chemotherapy-only regimens are being implemented for treatment of brain tumors and as conditioning regimens in BMT. Whether these changes will permit long-term survivors to experience normal growth remains to be seen.

## MUSCULOSKELETAL AND RELATED TISSUES

Functional and cosmetic disabilities involving bone, teeth, and muscle and other soft tissues are common and are reported in up to one-third of survivors of various pediatric cancers, notably solid tumors.[2] Most clinically significant problems involve bony abnormalities, such as scoliosis, atrophy or hypoplasia, avascular necrosis of bone, and osteoporosis. Scoliosis is a delayed consequence of radiotherapy to segments of the spinal column. As such, it is seen almost exclusively in patients with solid tumors.[30,40] The concavityof the deformity, which invariably occurs on the side of irradiation (Fig. 49-2), worsens during the adolescent growth spurt, irrespective of the age of the patient at radiotherapy. Nonetheless, in contrast to what had been observed after orthovoltage therapy, today's cases of scoliosis usually are not severe enough to require orthopedic intervention.

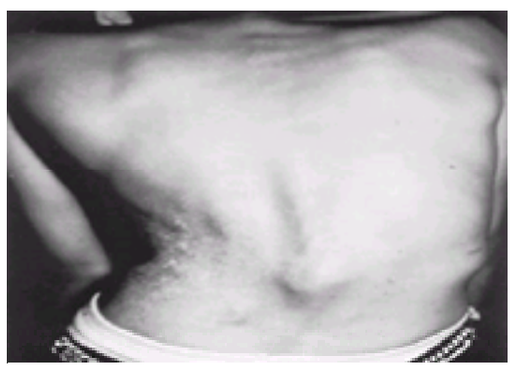

**FIGURE 49-2.** Kyphoscoliosis in a 15-year-old patient treated 14 years previously with 3,000-rad abdominal radiation for a left-sided Wilms' tumor.

In addition to causing scoliosis by direct effects on vertebral growth, abdominal irradiation for Wilms' tumor may result in hypoplasia of the ilium and atrophy of muscle, soft tissue, and skin within the field. These fibrosed tissues have been said to “act as a bow string across the vertebrae,”[30] increasing the degree of curvature. With better staging systems, the number of children requiring vertebral or paravertebral radiotherapy for Wilms' tumor or rhabdomyosarcoma has decreased. In addition, with current techniques, including symmetric irradiation of the entire spine, which spares large volumes of the adjacent soft tissue, as in the treatment of medulloblastoma[27] or as in total nodal irradiation for Hodgkin's disease, the risk of scoliosis is slight. Other factors that may contribute to the development of scoliosis include vertebral changes from metastatic tumor, laminectomy, and osteoporosis.[41] Kyphosis occurs less frequently and rarely in the absence of scoliosis.

Atrophy or hypoplasia has been reported to follow irradiation to most other bones, including the long bones of children with soft tissue sarcomas of an extremity[30,42] and the facial skeleton.[12,43] Probably because most patients already have achieved their maximum growth at the time of diagnosis, leg length discrepancy does not appear to be a significant problem in Ewing's sarcoma, in which the entire bone may receive as much as 7,000 cGy.[42]

Avascular necrosis of bone and osteoporosis are radiographic diagnoses and may be asymptomatic until the involved bone is subject to fracture or infection. Clinically significant avascular necrosis presenting as pain has been described most extensively in adults with head and neck tumors and in adults and adolescents with Hodgkin's disease and non-Hodgkin's lymphoma, in whom the incidence has been approximately 3%.[44] In the latter group, avascular necrosis most commonly involves the femoral heads, where it may be accompanied by slipped capital femoral epiphysis, but it has been described in virtually all locations and may be multifocal.

Although necrosis may develop during therapy, it is detected most commonly after therapy, and the latency period has been described to be as long as 13 years. Most avascular necrosis has been attributable to the direct effects of radiotherapy or the systemic effects of corticosteroids. Although these effects appear to show dose dependence, necrosis has been reported after cumulative prednisone doses as low as 500 mg.

In pediatric patients, the use of dexamethasone instead of prednisone during induction, reinduction, or delayed intensification has resulted in a growing number of reports of symptomatic avascular necrosis.[45,46,47,48] and [49] Among patients treated on a modified bendroflumethiazide protocol with 4 weeks of dexamethasone during reinduction, 4.4% developed symptomatic and often multifocal avascular necrosis. No cases of osteonecrosis were found in patients who did not receive dexamethasone.[48] Adolescents appeared to be at higher risk. This may, however, be a function of dose intensity, as recent trials randomly choosing between equivalent doses of dexamethasone and prednisone have not shown striking differences in rates of avascular necrosis.[47] In addition to radiotherapy and steroids, avascular necrosis in cancer patients anecdotally has been associated with single-agent cyclophosphamide or methotrexate and with cyclophosphamide in combination with methotrexate and 5-fluorouracil. Patients who develop symptomatic osteonecrosis are at high risk for debilitating orthopedic pain and may require joint replacement.

Like avascular necrosis, osteoporosis and osteopenia also have been related to steroids and to radiotherapy.[42] The association with methotrexate therapy is far more compelling than for avascular necrosis. Furthermore, methotrexate-induced osteoporosis and osteopenia appear primarily during therapy, often are associated with bone pain and occasionally with pathologic fractures, and usually resolve after methotrexate has been discontinued.[50] One study suggested that osteopenia, as documented by qualitative computed tomographic (CT) scans, may be related more to prior cranial irradiation than to the type of chemotherapy.[51] After radiation to the head in doses of less than 2,500 cGy, the degree of osteopenia may be significant enough to cause spontaneous fractures but may go undetected by plain radiographs. The development of this complication does not appear to correlate with effects on pubertal maturation. In other settings, both male and female survivors have been shown to have reduced bone mineral density, with a correlation between incidence (as high as 100%) and treatment-related gonadal failure.[52,53] Poor calcium intake and increased body weight may be other predictive factors.

Other bone changes common after irradiation are exostoses; asymptomatic roentgenographic findings that include growth-arrest lines; lines of increased density parallel to end plates (known as *Park lines*), and epiphyseal irregularities.[54] In general, these changes are seen after 2,000 cGy or more given in 150- to 200-cGy fractions. As in scoliosis, these changes are seen primarily in patients who were still growing at the time of therapy and, therefore, have been reported most commonly

in survivors of Wilms' tumor, Hodgkin's disease, neuroblastoma, and medulloblastoma.

The contribution of irradiation of soft tissue to the development of scoliosis has been noted. In addition, irradiation may produce disfiguring soft tissue (including breast) hypoplasia and pigmentary changes even without functionally significant abnormalities of neighboring bone ( Fig. 49-3). Other connective tissue abnormalities seen in long-term survivors of childhood cancer include the following: scarring and contractures resulting from extravasation of drugs, such as vincristine, dactinomycin, and anthracyclines; edema resulting from irradiation- or tumor-related lymphatic obstruction; nasolacrimal duct obstruction related to radiation fibrosis; chronic conjunctivitis, also caused by radiation[42]; various degrees of alopecia; the sclerodermatous skin manifestations of GVHD; one or more components of the sicca syndrome (dry eye, dry mouth) secondary to GVHD or to radiotherapy involving the lacrimal or salivary glands; and cataracts (see the section Neuropsychological and Neurologic Function ). The bony and soft tissue complications of amputation and endoprostheses are discussed elsewhere (see Chapter 32 and Chapter 35).

**FIGURE 49-3.** Progressive pectoral muscle hypoplasia after radiation to a posterior mediastinal neuroblastoma. The child was 10 months old at the time of diagnosis. **A**, **B**, and **C** were taken at 2, 4, and 6 years, respectively, after radiation. Hypoplasia of the ipsilateral breast can be anticipated.

Dental and maxillofacial abnormalities, although a composite of radiation effects on bone and soft tissue, bear separate mention. Delayed or arrested tooth development has been reported after 1,800 cGy or more to the cranium in children with ALL and to the neck or entire mantle for Hodgkin's disease using megavoltage equipment.[55,56] Severe structural tooth abnormalities, including malocclusion and caries, are seen almost uniformly after 4,500 to 6,500 cGy to ports involving the oronasopharyngeal growth center of the lower face. The effects of irradiation (including TBI) on dentition are most severe when this therapy is administered to children younger than 6 years.[33,43] Chemotherapyalso appears to contribute to cavity formation and gingivitis and to the development of cosmetically significant grooves, pits, and discoloration.[57]

Detection and diagnosis of musculoskeletal and connective tissue toxicities are largely a matter of suspecting these problems in vulnerable hosts, of recording a careful history, and of performing a thorough physical examination. Particularly when patients are at high risk for the development of scoliosis, follow-up visits should be scheduled every 6 months during their adolescent growth spurt. Although soft tissue or bony asymmetries may be obvious, mild pain or a history of fractures may be the only indication of avascular necrosis or osteoporosis. The need for diagnostic radiographs and appropriate referral in the case of clinically apparent disease is obvious (Table 49-2). The relative risk-benefit ratio of “routine” radiographs of bones encompassed by radiation ports and of bone densitometry is less clear. However, because of progress with various interventions (including the use of calcium supplementation, calcitonin, bisphosphonates, and sex hormone replacement in postmenopausal patients), such studies can be recommended every few years, particularly in patients who are at least 5 years from diagnosis. Optimally, dental evaluation should be performed yearly or every other year for life. Scrupulous oral hygiene, including flossing and fluoride supplementation, helps to reduce the dental abnormalities.

## NEUROPSYCHOLOGICAL AND NEUROLOGIC FUNCTION

Among survivors of all types of pediatric cancer, the incidence of significant neuropsychological and neurologic abnormalities is variable, depending on tumor type, location, and timing and method of CNS treatment. For children who have brain tumors[3,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75 and 76] or acuteleukemia[77,78,79,80,81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100,101,102,103 and 104] and in whom neuropsychological and neurologic sequelae have been studied most extensively, disabilities necessitating special education or even institutionalization have been reported in as many as 8% to 50% (see Chapter 19 and Chapter 27). For example, in one report of 36 children who had various intracranial tumors and had survived 5 years after treatment, 45% had intelligence quotients (IQs) of less than 90, and 17% had IQs of less than 70.[3] Patients who had other tumors and required specific CNS treatment appear to be at similar risk.

Radiotherapy is the most problematic for growth. Learning difficulties among childhood cancer survivors primarily have been attributed to cranial irradiation and are related to cumulative dose, size of individual fraction, and age at time of treatment. [3,58,59,60,61,62,63,64,65,66,67 and 68,76,77,78 and 79] The impairment may be subtle or devastating but usually is not progressive after the first 3 to 5 years after radiotherapy. [64,65,79] Children having brain tumors who are younger than 36 months at the time of diagnosis and who have been treated like older brain tumor patients with 3,500 to 5,500 cGy are at highest risk for development of serious cognitive impairment and such neurologic problems as blindness and visual impairment. [58,59 and 60] In one study, 21 of 28 children who received diagnoses before the age of 36 months were assessed at an average of 8 years after treatment. Significant impairment in neuropsychological performance and developmental outcome was found in most of the 7 patients who were treated with cranial irradiation (mean IQ, 80), as compared with the 14 who received only surgery or chemotherapy (mean IQ, 97). [62] Delaying radiotherapy until affected patients are older has been achieved in some patients with good results. [61,63] With lower doses of radiation that now can be used safely because of the antitumor effectiveness of chemotherapy,[66] the neurologic morbidity is expected to be significantly less than in the past. Early results support this prediction.[67]

In children with brain tumors, factors other than irradiation and age and possibly at least as influential in dictating neurologic outcome include tumor location (patients with brainstem, hypothalamic, and fourth ventricular tumors being at highest risk for neuropsychological deficits), obtundation at diagnosis, hydrocephalus, need for permanent shunting, and postoperative complications.[68,70,76]

Most children with brain tumors treated with surgery and chemotherapy have little or no permanent adverse neurologic sequelae as a result of the treatment. Among 20 children with brain tumors treated with surgery and chemotherapy before age 3, the academic and IQ scores, at an average of 6 to 7 years after treatment, were the same as in healthy children, despite the patients' treatment at such a young age. [62]

Radiation dosages in children with ALL generally are lower than those used for treatment of brain tumors and, on average, the extent of impairment is less. However, the neuropsychological performance of those who received cranial irradiation are significantly lower as compared with patients who had ALL and received only chemotherapy, their healthy siblings, the population at large, or cancer patients not receiving CNS therapy of any type. [77,78,80,81,82,83,84,85,86,87 and 88] When comprehensive assessments are conducted, specific areas of impairment have been identified, rather than global decreases in IQ, notably in attention capacities and other nonverbal cognitive processing skills. [89,90] These focal deficits, which have been shown to correlate with CT and magnetic resonance imaging (MRI) abnormalities of the brain,[91,92] have been postulated to be due to an underlying radiation-induced attention deficit. Further support for the hypothesis was found in an electrophysiologic study of an attentional disorder underlying the poorer performance of irradiated patients as compared with nonirradiated patients on neurobehavioral tasks. Evoked potential measures indicated cortical disorganization and slowing during mental tasks in the group of patients who had undergone radiotherapy. [93] Several studies suggest that girls are more likely than boys to experience a decline in IQ after cranial irradiation and intrathecal therapy or after systemic high-dose methotrexate.[94,95,96,97 and 98] These gender differences variably have been demonstrated with respect to performance IQ or verbal IQ. [82,83] Although it is not possible to certify that CNS prophylaxis affects one gender more than the other, girls may be more vulnerable than boys to cranial irradiation by virtue of their more rapid brain growth and development during early childhood. [95]

As for patients with brain tumors, age at the time of CNS preventive therapy for ALL is likely to be an important factor in resulting deficits. [87,88 and 89,95,96,97,98,99 and 100] Most investigators agree that children younger than 5 at the time of prophylaxis are at risk of neurocognitive deficits higher than that of older children. One of the

better-designed studies comparing children in three diagnostic age groups did find that those who were younger than 3 at the time of treatment showed the greatest deficiencies.[99] Conversely, at no age are patients completely safe from neurobehavioral sequelae, which have been described even in adults. [105]

In recent years, dose reduction of cranial irradiation from 2,400 to 1,800 cGy has been instituted for CNS prophylaxis, in an effort to decrease neurologic sequelae. Although early results suggested that cognitive effects were the same as those observed in children who had received 2,400 cGy, [79] recent reports suggest that those children treated with the higher dose have more significant late abnormalities than do those who received 1,800 cGy. [78] However, possibly the time to onset is longer after lower doses of radiotherapy, and additional follow-up will be needed to be sure of the extent of low-dose irradiation-induced sequelae. [79]

Based on a number of retrospective and longitudinal studies, children with ALL treated with chemotherapy alone (systemic and intrathecal methotrexate), without cranial irradiation, are not more likely to suffer severe adverse effects than are children who had tumors and received no CNS treatment. [77,83,84] However, other studies have suggested that such patients experience declines in neuropsychological function comparable to those seen in patients treated with 1,800 cGy cranial irradiation.[86] As compared to those treated with single-agent intrathecal methotrexate, patients who received triple intrathecal therapy without cranial irradiation for CNS prophylaxis have a slightly higher risk of cognitive impairment. [86]

An intriguing question is whether the deficits seen in children with ALL after CNS preventive therapy are not only an effect of radiotherapy but a reflection of an adverse interaction between cranial irradiation and the accompanying intrathecal or systemic methotrexate Although one study addressing this possibility disproved a synergistic effect of cranial irradiation and intrathecal methotrexate, [101] these and other investigators have suggested a possible additive effect of cranial irradiation with intrathecal methotrexate.[95,102,103]

At the extreme end of the spectrum of chronic neurotoxicity associated with CNS treatment is progressive necrotizing leukoencephalopathy. It is characterized clinically by dementia, dysarthria, dysphagia, ataxia, spasticity, seizures, and coma, and histopathologically by reactive astrocytosis, gliosis, and demyelinization. [106] Blindness also has been described, apparently because of involvement of the optic chiasm. [107] As with learning disabilities, the more severe manifestations have been described most frequently in patients treated with a combination of intrathecal or intraventricular and systemic methotrexate and with at least 2,400 cGy of cranial irradiation, particularly in children. Leukoencephalopathy also has been reported in several patients after BMT when preparatory regimens included 1,000 cGy TBI. [32] A less common neurotoxicity in long-term survivors is radionecrosis. This entity, which has been described for as long as 12 years after 5,000 to 6,000 cGy, presents as increased intracranial pressure with focal findings. An intracranial mass with surrounding edema is characteristic on CT scan. [68] Irradiation-induced cerebral vasculopathy presenting radiographically as moyamoya syndrome (abnormal netlike vessels and transdural anastomoses) and clinically as ischemic events also has been described years after irradiation for intracranial tumors. [69] (Neurotoxicity associated with GVHD is reviewed in Sanders.[32])

At the other end of the spectrum are the electroencephalographic and CT or MRI brain scan abnormalities reported in children with ALL or head and neck tumors.[108,109,110] and [111] The latter changes include intracerebral calcification, dilatation of the ventricular and subarachnoid spaces, and a decreased parenchymal attenuation coefficient (white-matter hypodensity). In one series, changes were seen only in children who had been younger than 8 years at diagnosis. [109] In a comparative study of CT scans of patients given one of three methods of CNS prophylaxis, although similar minor abnormalities were seen after intrathecal methotrexate alone, intrathecal methotrexate plus intermediate-dose (500 mg per $m^2$) methotrexate, and intrathecal methotrexate plus 2,400 cGy of cranial irradiation, significant CT abnormalities were found only in the last group. [110]

How neuropsychological deficits correlate with CT changes is not clear. In one study, however, patients with intracerebral calcification (particularly of the basal ganglia) or cortical atrophy demonstrated subtle impairment of attention and verbal memory. [91] These data suggest that CT scan abnormalities presage clinical deficits, a speculation that is especially worrisome in light of the finding that calcifications first may appear more than 7 years after CNS prophylaxis. [109]

CT scans of the spinal cord have not been done systematically. Although autopsy findings have shown that the pathologic changes of leukoencephalopathy also can affect the spinal cord (subacute necrotizing leukomyelopathy), this does not seem to be a symptomatic problem in long-term survivors of cancer. In patients with solid tumors treated with high doses of radiation (usually greater than 5,000 cGy), particularly to the head and neck or brachial plexus, cranial and peripheral neuropathies have developed over months or years as a result of axon necrosis or fibrosis. [112,113] Blindness secondary to radiation-induced necrosis of the optic nerve has been described even at doses as low as 1,000 cGy.[114] Sensorimotor neuropathies arising during therapy with vinca alkaloids can persist, although for the most part these effects are reversible and therefore are not seen as late effects of therapy. Although the same can be said for high-dose cytosine arabinoside, cisplatin, and ifosfamide, long-term follow-up data are limited.

Additional neurologic residua include hearing loss from therapy with cisplatin [115] or aminoglycosides or from the chronic otitis media of histiocytosis X (Langerhans' cell histiocytosis) and head and neck rhabdomyosarcoma.[11,42] Blindness from enucleation or radiation-induced cataract formation may occur in patients with retinoblastoma, soft tissue sarcomas, or other tumors involving the orbit. Less extensive visual impairment may result from steroid-induced or radiation-induced posterior capsular cataracts in patients with ALL after cranial irradiation or in patients receiving bone marrow transplants. [32] Other neurologicor neuroendocrine abnormalities may persist in disease-free survivors of childhood cancer and presumably are related to the underlying disease rather than to therapy. These include the ataxia-opsoclonus-myoclonus syndrome seen occasionally in children with neuroblastoma, and diabetes insipidus in patients with histiocytosis X.

As suggested in this section, many patients followed up by pediatric oncologists are at risk for late CNS toxicity and therefore must be monitored carefully. Evaluation of learning disabilities usually is pursued by the school system when affected children's grades are poor (see Chapter 50). However, because lesser abnormalities may go undetected and still may interfere with optimal learning, neuropsychological screening should be performed routinely, at least in children who have received cranial irradiation and especially in those who were younger than 8 years at the time of diagnosis. An age-standardized battery of tests should be used to measure intellectual ability, visual and somatosensory perception, visuomotor and motor skills, language, memory and learning, academic achievement, behavior, and social functioning. Because CNS toxicity is not static, repeating neuropsychological tests every 2 to 3 years is appropriate until early adulthood. Test results then can be used to individualize remedial instruction.

Some oncologists make the same recommendations with respect to serial CT or MRI scans. The expense and uncertain meaning of many of the CT abnormalities lead us to recommend that these scans be reserved for children whose deficits are detected on psychometric testing or who have other evidence of leukoencephalopathy. Screening for hearing deficits is particularly important after treatment with cisplatin, as deficits may occur unpredictably during or after therapy, even when monitoring audiograms have been normal. Although various cerebrospinal fluid proteins, including myelin basic protein, have been proposed as predictors of CNS damage, their use is controversial and is not recommended on a routine basis. Care should be taken to explain to affected parents and children that some school problems may, in fact, be related to treatment. The magnitude of neurologic sequelae emphasizes the need to develop alternative approaches to their prevention and therapy. Refinements in irradiation techniques, the elimination of irradiation altogether in selected lower-risk patients, and prophylactic or therapeutic pharmacologic interventions, such as with methylphenidate, are under investigation. [116]

## GONADAL FUNCTION

### Male Patients

Both germ cell depletion and abnormalities of gonadal endocrine function have been seen in male survivors of cancer. Most commonly, these changes have been thought to result from therapy—radiotherapy, surgery, or chemotherapy—with specific late effects differing as a function of age at diagnosis. The effects of testicular irradiation on germ cell number have been well studied. Reduced sperm production occurs in a dose-dependent manner with fractionated exposures of 10 to 600 cGy.[117,118] and [119] In patients with seminoma treated with para-aortic and ipsilateral pelvic irradiation after unilateral orchiectomy, incidental doses of 32 to 178 cGy to the remaining testicle have been associated with azoospermia resolving over 6 months to 2 years. [119] Among men who had Hodgkin's disease and received inverted-Y irradiation and who, despite lead shielding of the scrotum, were estimated to have received a cumulative dose of 140 to 300 cGy to both testes, 100% developed azoospermia without recovery after 2 to 40 months of follow-up.[120] Although detailed dosimetry was not reported regarding scatter to the testes from ports encompassing soft tissue sarcomas of the thigh or abdomen, irradiation has been shown to contribute to the 100% incidence of azoospermia in that setting. [121] At doses of 400 to 600 cGy, azoospermia may persist for 3 to 5 years; at doses in excess of 600 cGy, germinal loss with resulting increases in follicle-stimulating hormone (FSH) and decreases in testicular volume usually appears irreversible. In one study, azoospermia was universal in a large group of male patients who were past puberty at the time of TBI (1,000 cGy single fraction). Only 2 of 41 patients had recovery of sperm production 6 years after BMT. [32]

Prepubertal testicular germ cells also appear to be radiosensitive, although assessing tubular damage may be difficult until affected patients have gone through puberty. In a follow-up of ten young men (ages 17 to 36) who had received an estimated 268 to 983 cGy scattered from irradiation to a Wilms' tumor during childhood, oligospermia or azoospermia was found in eight survivors. [122] A similar group of eight patients who still were prepubertal at the time of evaluation did not yet show intermittent elevations of FSH, although presumably they, too, had depleted germinal epithelium. Data on gonadal function in boys treated with irradiation alone for Hodgkin's disease[123] or with TBI for acute myeloid leukemia[124] before puberty are limited, but they are consistent with data from older patients. Transplantation of the testes to the thigh or abdomen during irradiation appears to spare the prepubertal testis. [125]

Radiotherapy also may be toxic to Leydig cells, although at doses higher than those that are toxic to germ cells. As summarized by Sklar, [126] Leydig cell damage is dose-dependent and inversely related to age at treatment. Boys treated prepubertally or peripubertally with 2,400 cGy for testicular leukemia, in addition to experiencing germ cell depletion, are at high risk of delayed sexual maturation associated with decreased testosterone levels despite increased luteinizing hormone (LH) levels.[127,128,129 and 130] Of 16 boys with hematologic malignancy treated prepubertally with TBI, 11 experienced delayed onset of puberty. [32] Fractionated doses of less than 1,200 cGy to the prepubertal testis are compatible with normal pubertal maturation in most patients, although often at the expense of compensated Leydig cell failure (normal testosterone levels with elevated LH levels). [126] Cranialor craniospinal irradiation itself, as in children with ALL [131] or primary brain tumors other than lesions involving the hypothalamic or pituitary glands, [132] does not seem to damage testicular function, although precocious onset of puberty has been reported. [133] Adolescent and young adult male patients are relatively radioresistant, and fractionated doses greater than 3,000 cGy may induce Leydig cell failure in 50%. [134]

Chemotherapy also can interfere with testicular function. In particular, various alkylating agents and the methylhydrazine procarbazine decrease spermatogenesis in long-term survivors of cancer. Although many of the data come from patients with nephritis, studies of adults with non-Hodgkin's lymphoma and soft tissue sarcomas have confirmed that the effects of cyclophosphamide and chlorambucil are dose-dependent but reversible in up to 70% of patients after therapy-free intervals of several years.[121,135,136,137 and 138] Among pubertal or adult male patients treated for Hodgkin's disease with five or six cycles of mechlorethamine, vincristine, prednisone, and procarbazine (MOPP) and evaluated 1 to 2 years after completion of therapy, azoospermia is found in 80% to 100%. [138,139,140 and 141] However, this effect is reversible in only some 20% of cases even 7 years after therapy, a percentage that may increase with fewer MOPP cycles and may decrease with pelvic irradiation. [142] After doxorubicin(Adriamycin), bleomycin, vinblastine, and dacarbazine (ABVD), the incidence of azoospermia appears to be lower (36%) and the incidence of recovery higher (100%) than after MOPP.[143]

Although evaluation of testicular histology and hormonal status in boys shortly after treatment for ALL with cyclophosphamide, cytosine arabinoside, or both has suggested that germ cell depletion occurs as an acute toxicity in all age groups, [144] long-term follow-up of patients who received single-agent cyclophosphamide for nephritis or as the pretransplantation preparatory regimen for aplastic anemia [145] suggests that prepubertal testicular germ cells may be relatively resistant to chronic toxicity. Nonetheless, damage to spermatogenesis in the developing testis may be severe and permanent. In one follow-up of 13 boys who were older than 16 at the time of evaluation and who had been treated prior to puberty with MOPP alone (1 to 12 cycles), the majority had small testes, and all were either azoospermic or severely oligoospermic more than 4 years off therapy.[146] Small testes and mild increases of FSH have been observed in another series of boys several years after prepubertal treatment with MOPP for Hodgkin's disease[123] and after nitrosoureas for brain tumors[132] or cyclophosphamide for paratesticular rhabdosarcoma.[125] Azoospermia or oligospermia is common in survivors of childhood or adolescent ALL treated on bendroflumethiazide regimens. [147]

In contrast to their prominent effects on germ cell epithelium, chemotherapeutic effects are less striking on slowly dividing Leydig cells, and those effects that are seen tend to be age-related. After MOPP in prepubertal boys, normal pubertal progression and normal adult levels of testosterone are the rule; gynecomastia with low testosterone and increased LH have been reported in patients treated during adolescence; compensated Leydig cell failure (increased LH with low normal testosterone levels or exaggerated FSH and LH responses to LH-releasing hormone) without gynecomastia is common in adults. [138,139 and 140] Compensated Leydig cell failure has been found at the time of diagnosis in almost one-half of men with Hodgkin's disease. [148] Decline in libido without impotence may be a common problem and does not correlate with testosterone levels.[149] The reversibility of these abnormalities has not been addressed in the literature. Increased [149] and normal[7,139] levels of prolactin have been described in male patients years after receiving MOPP or other regimens. However, the relationship of these findings to serum testosterone levels or the presence of gynecomastia is not clear.

Compensated Leydig cell failure sometimes associated with gynecomastia also has been described after cisplatin-based therapy in adults. [149] Other combination-chemotherapeutic regimens, including prednisone, vincristine, methotrexate, and 6-mercaptopurine as used in adolescent boys with ALL [131] and high-dose methotrexate and vincristine used in men with osteosarcoma,[150] appear to cause either no or only transient gonadal toxicity. Whether the even higher doses of chemotherapeutic agents included in current protocols for high-risk ALL will be as benign remains to be seen.

The effects of surgery on the gonads include impotence or retrograde ejaculation after bilateral retroperitoneal lymph node dissection [125,151] or partial or complete pelvic exenteration. In patients with primary CNS tumors involving the hypothalamus or pituitary, surgical resection may cause secondary hypogonadism. [152] Hydroceles have been seen in long-term survivors of Hodgkin's disease, [153] Wilms' tumor (unpublished observation), and paratesticular rhabdosarcoma [125] after retroperitoneal surgery or radiotherapy.

The gonadal toxicities listed in this section, although not life threatening, are of serious concern to patients and their families, particularly in the case of young men who have not had children already at the time of diagnosis. This concern has popularized pretreatment sperm banking, sometimes even from prepubertal boys. Cryopreserved semen from adults has, in some cases, produced normal children.[154] The value of this precaution has been questioned, however, because many men with Hodgkin's disease, primary testicular cancer, and diverse metastatic cancers have lower-than-expected sperm counts and decreased sperm motility at the time of diagnosis, regardless of stage of disease or the presence of B symptoms (see Chapter 23).[148,155,156 and 157] Even when semen samples appear adequate, the expense of sperm banking (which frequently is not covered by insurance), the variable quality control among sperm banks, and concern regarding viability and integrity of sperm stored for long periods make this technique controversial.

Patients should be screened routinely for problems of gonadal function. This screen should include recording an age-appropriate history with specific attention to problems with libido, impotence, or fertility and examination for gynecomastia, Tanner staging of body hair, and penile and testicular size. Hormonal evaluation, including at least a single measurement of serum LH, FSH, and testosterone levels, should be performed in pubertal boys who are concerned about their gonadal development or fertility and in boys whose puberty appears to be delayed. Serum inhibin-B concentrations have been found to correlate with testicular size and FSH levels and have been recommended by some investigators.[158] Semen analysis may be helpful.

When abnormalities in gonadal function are detected, close cooperation with an endocrinologist is essential in planning hormonal replacement therapy or in monitoring patients for spontaneous recovery. When no abnormalities are noted on history and physical examination but sexual maturity has not been completed, these studies should be repeated every 1 to 2 years.

The value of trying to prevent gonadal dysfunction is obvious. It is hoped that the use of such chemotherapeutic regimens as ABVD (see Chapter 21) will be less toxic but as effective and that irradiation techniques will be refined, as has been mentioned. Extensive surgical procedures have been made nearly obsolete by intensive multimodal approaches.

**Female Patients**

In contrast to what is seen in boys and men, germ cell failure and loss of ovarian endocrine function usually go hand in hand and together may be the result of radiation damage. Manifestations are both age- and dose-dependent. Irreversible ovarian failure (primary or secondary amenorrhea, increased LH and FSH levels with or without symptoms of menopause, and loss of libido) is an almost universal result of 400 to 700 cGy conventionally fractionated and delivered to both ovaries (as in whole-abdomen irradiation) in women older than 40.[159] Prepubertal ovaries are relatively radioresistant; despite higher doses (1,200 to 5,000 cGy), primary amenorrhea and delayed puberty eventually occurred in only 68% of patients treated at a mean age of 6.9 years. [160] In one series, only 23% of prepubertal and adolescent girls in whom at least one ovary was at the edge of the radiation field (and therefore had received a dose of 90 to 1,000 cGy) had ovarian failure. [161] When secondary amenorrhea results from such modest doses, which are similar to those administered to the ovaries after midline oophoropexy and lead shielding with pelvic irradiation in Hodgkin's disease, it appears to be reversible within several months to 4 years in 50% to 60% of patients. [161,162] In some series, TBI (1,000 cGy single fraction) has been associated with primary amenorrhea and absent secondary sexual characteristics in most patients treated as young girls and followed up for as long as 10 years.[32,124] However, others have reported normal pubertal progression, although with elevated FSH levels, after TBI during early childhood. [163,164]

Premature menopause also has been reported.[124]

The effect of craniospinal irradiation is less clear. In one report, girls with medulloblastoma treated with craniospinal irradiation alone (3,000 cGy to the spine) did not experience gonadal toxicity.[132] In contrast, among similarly aged girls with ALL, those receiving craniospinal irradiation (1,800 to 2,400 cGy) or craniospinal plus abdominal irradiation (1,200 cGy) experienced incidences of increased FSH and LH of 49% and 93%, respectively, and a large proportion of these children had delayed menses.[165] A recent study of 38 women who had been treated with cranial irradiation for childhood ALL suggested that polycystic ovaries are found in more than 20% after 1,600 to 1800 cGy and in more than 80% after more than 2,000 cGy.[166]

Ovarian failure also has been associated with chemotherapy. Although the morbidity is less than in male patients, single alkylating agents (cyclophosphamide, busulfan, L-phenylalanine mustard) and MOPP are the best-described culprits.[167,168,169 and 170] As with radiotherapy, toxicity is dose- and age-dependent. Women older than 40 may develop amenorrhea with only 1 to 4 cycles of MOPP, as compared with 3 to 12 cycles in the 30% of women who are younger than 35 and later become amenorrheal. Those diagnosed before puberty treated with conventional doses of single alkylating agents or MOPP generally are capable of normal puberty,[140,169] although they may have transient clinical evidence of ovarian failure.[132,171,172,173,174 and 175] On the basis of results of the Five Center Study, the incidence of premature menopause in women who had been treated prior to puberty is not greatly in excess of that in controls.[174] As with male patients, treatment of girls with prednisone, vincristine, methotrexate, and 6-mercaptopurine[169] or of women with vincristine and methotrexate[150] has not been associated with gonadal dysfunction. After myeloablative doses of alkylating agents, permanent ovarian failure can be expected at all ages.[145]

The diagnostic evaluation of ovarian dysfunction rests on history (primary or secondary amenorrhea, menstrual irregularity, and pregnancies or difficulties becoming pregnant); Tanner staging of breast and genital development; and serum gonadotropin and estradiol levels. These studies may be repeated at intervals similar to those suggested for male persons. Because young women may be destined for early menopause, however, they should have long-term follow-up. In addition to investigating the foregoing approaches to prevention of gonadal failure, some researchers have begun to examine the use of oral contraceptives to suppress ovarian function, thereby rendering the ovaries resistant to the effects of chemotherapy.[175] The harvesting and freezing of ova prior to therapy that is likely to cause premature ovarian failure are under investigation.[176]

For patients who are fertile after anticancer therapy, concerns remain about the ability to have normal pregnancies and normal children. Demonstrating the potential teratogenicity or mutagenicity of modern anticancer therapy in patients is difficult for several reasons. Few patients have children during or after treatment, and many who conceive elect to have an abortion. Moreover, abortuses and live-born infants have not been scrutinized for defects in morphogenesis, growth, and development. Differences in radiotherapeutic or chemotherapeutic drug combinations and dosages render correlations difficult. Finally, the introduction of new therapeutic agents has thwarted the accumulation of sufficiently similar cases from which to draw definitive conclusions. Nonetheless, enough case reports exist to suggest a real risk of congenital or developmental abnormalities in the offspring or spontaneous abortuses of patients who have been given intrapartum chemotherapy. The risk appears to vary with the therapeutic regimen and, especially, with the timing of the pregnancy with respect to drug exposure.

On the basis of available information, pregnancy outcome is probably most threatened by chemotherapy given during the first trimester. Chemotherapy appears less commonly, if ever, to have caused serious abnormalities when used later in pregnancy. A detailed summary of the effects of anticancer agents administered during pregnancy is reported elsewhere.[177]

Whether therapy completed before pregnancy is a risk to subsequent offspring is a problem more relevant to the long-term survivor of childhood anticancer therapy. As recently reviewed,[178] numerous series and case reports have suggested that intensive chemotherapy completed before pregnancy, including myeloablative chemotherapy prior to BMT,[179] is compatible with normal offspring. A recent report from the Childhood Cancer Survivor Study (CCSS) of 6,017 successful pregnancies in 2,978 five-year survivors of childhood cancer (1,151 male, 1,827 female) did not identify excess adverse outcomes for most chemotherapeutic agents.[180] A tentative association was drawn between procarbazine in cumulative doses greater than 5,000 mg per $m^2$ to male patients and miscarriage rates in their partners. Although in this and other series, both major and minor anomalies were observed, they did not appear to occur in excess of that seen in the population at large.

Tentative associations between dactinomycin exposure and cardiac malformations in offspring of female survivors[181] and between cyclophosphamide and birth defects[182] have not been borne out. One study in which the survivors' children were examined at ages 0 to 12 (median, 2.5 years) did not detect any significant malformations, problems with school performance, or excess of minor abnormalities after various intensive combinations commonly used in the treatment of leukemia, Hodgkin's disease, and sarcomas.[183] A report of an unusually low son-daughter ratio was interpreted as suggesting a deleterious effect of chemotherapy on germ cells,[184] but larger series have not reproduced this observation.[180]

Although chemotherapy has received most of the attention, irradiation involving the ovaries also may compromise pregnancy outcome. Reports document an increased risk of perinatal death, prematurity, and low birth weight in the offspring of female long-term survivors,[184] especially those of Wilms' tumor[185] and Hodgkin'sdisease,[186] whose therapy included abdominal or pelvic irradiation. In the latter series, as compared with women with Hodgkin's disease treated with chemotherapy alone, women who had received both chemotherapy and radiotherapy were found to have a significantly higher incidence of abnormal offspring. Moreover, wives of men with Hodgkin's disease treated with both chemotherapy and radiotherapy—but not those treated with chemotherapy alone—appeared to have an increased incidence of spontaneous abortions.[186] These observationshave not been reproduced in larger series.[180] Multivariate analysis has suggested a correlation between a diagnosis of CNS tumor (independent of cranial or craniospinal irradiation) and risk of miscarriage, suggesting that some central factor may be important for sustaining pregnancy.[180]

The possibility of mutagenic (as opposed to teratogenic) effects of anticancer therapy also has been raised. One cytogenetic study showed nonclonal chromosomal abnormalities in peripheral blood lymphocytes of six of nine long-term survivors of childhood ALL a median of 7 years after therapy, a finding suggesting that genetic damage can be sustained, at least by somatic cells.[187] However, accumulating studies of several thousand offspring of long-term survivors who had been treated during childhood or adolescence with chemotherapy with or without radiotherapy have failed to demonstrate an increased overall risk of childhood cancer in offspring of childhood cancer survivors[178,188,189] (CCSS preliminary data). One subset of patients, those whose own cancers are due to a genetic predisposition, may have children with the same predisposition to malignancy. The genetics of embryonal tumors, such as retinoblastoma or Wilms' tumor, and the risks of malignancy for patients with inherited nonmalignant conditions, such as von Recklinghausen's disease, are discussed elsewhere (see Chapter 3).

Patients interested in having children after the completion of therapy should do so, although reasonable advice is for them to wait 1 year or more to be more certain that they are disease-free. Difficulties in becoming pregnant can be evaluated (as described). Because little is known about the problems of children born to survivors of childhood cancer, long-term general follow-up should be emphasized. The importance of optimizing the accrual and dispersal of information about pregnancy outcome in patients with a history of cancer cannot be overemphasized. A central registry is in place at the Children's Hospital of Oklahoma (405-271-8685).

## THYROID FUNCTION

Hypothyroidism is the most common nonmalignant late effect involving the thyroid gland and almost always is due to radiation to the neck for nonthyroid malignancy. At a mean of 7 years (1.5 to 16.0 years) after radiation doses of 1,500 to 7,000 cGy, laboratory evidence of primary hypothyroidism [increased serum thyrotropin (thyroid-stimulating hormone) with normal or low thyroxine ($T_4$) levels] has been demonstrated in 30% to 90% of patients with Hodgkin's disease, non-Hodgkin's lymphoma, and primary intracranial or head and neck tumors[5,9,190,191,192,193,194,195,196,197,198,199 and 200] and in as many as 50% of children after BMT for hematologic malignancy.[124] The likelihood of abnormalities depends on radiation dose, and children treated with less than 1,500 cGy have a less than 20% incidence of chemical hypothyroidism.[200] A recent questionnaire-based follow-up of 1,791 5-year survivors of childhood and adolescent Hodgkin's disease who had received a mean dose of 3,500 cGy to the thyroid has estimated that 28% have documented hypothyroidism.[198] The relative risk was 17 as compared with that of sibling controls. Within the pediatric age range, adolescents may experience more severe abnormalities with higher thyroid-stimulating hormone levels than those seen in young children.[190,198] In one series, 48% of patients who were younger than 20 years and had Hodgkin's disease had elevated thyrotropin levels, as compared with only 33% of older patients, a difference believed to be significant.[196] Other factors that may contribute to the development of hypothyroidism in survivors of cancer include female gender, hemithyroidectomy, and use of iodide-containing contrast material, as in lymphangiography. In some instances, hypothyroidism has been reversible after as long as 3 years even without replacement therapy.[193] Thyroid cancers (see the section Second Malignant Neoplasms), exophthalmos, and symptoms of hypothyroidism, including myxedema coma, have been reported in some of these patients. The risk of thyroid nodules has been reported to be 27-fold that of sibling controls occurring as a late event, with a mean diagnosis time of 14 years after treatment.[197,198] Female gender and radiation dose exceeding 2,500 cGy are independent risk factors.

Approximately 7% of these nodules have been found to be malignant.

Much lower doses of radiation also may carry a risk of primary hypothyroidism. In a study by the Children's Cancer Group, [201] thyroid function was evaluated in 175 children who had ALL and had received 2,400 cGy cranial irradiation and were at least 7 years from diagnosis. On the basis of dosimetric measurements, [202] these patients' thyroidswere estimated to have received as much as 180 cGy. Five patients (3%) had low serum $T_4$ and increased thyrotropin levels, and 11 others (6%) had normal $T_4$ levels at the expense of increases in thyrotropin, figures that appear to be in excess of those of the general population or of patients who had received only 1,800 cGy or intrathecal treatment alone (Rogers, *personal communication*). The absence of significant thyrotoxicity from antileukemic chemotherapy without irradiation has been substantiated by other investigators. [203] Similarly, evaluation of patients with Hodgkin's disease treated with MOPP without irradiation has not detected a significant incidence of thyroid dysfunction. [193] Whether other chemotherapy contributes to the development of hypothyroidism has not been studied. Secondary hypothyroidism with low thyrotropin and $T_4$ levels, although reported,[5,9] appears to be uncommon after irradiation to the head or neck.

Hyperthyroidism has been described in some 5% of patients after irradiation for Hodgkin's disease [192,198,204] orother nonthyroid neoplasms of the neck[205] and after preparation for BMT,[32] a relative risk of approximately eight as compared with results in sibling controls.

Patients who have received 1,000 cGy or more to the neck should be screened indefinitely by routine physical examination for thyroid abnormalities, as nodules may be late-appearing. Measurement of serum thyrotropin, $T_4$, and free $T_4$ levels should occur on a yearly basis for at least 7 years from the conclusion of therapy. Evaluation and treatment by an endocrinologist are recommended if any abnormalities are detected. Careful shielding of the thyroid during irradiation, elimination of radiation or the use of lower doses (as now advocated in some centers for patients with Hodgkin's disease), and avoidance of the concurrent use of radiation and iodide-containing contrast materials should help to decrease the incidence of thyroid abnormalities.

## CARDIAC FUNCTION

The intensive irradiation and chemotherapy necessary to improve event-free survival in childhood cancer patients may be associated with both acute and chronic effects on the heart. The “late” cardiotoxic effects seen in long-term survivors can include pericarditis, myocarditis, left ventricular failure, arrhythmias, coronary artery disease, myocardial infarction, heart failure, and even death.

The most well-studied cardiotoxins are the anthracycline antibiotics, doxorubicin (Adriamycin) and daunorubicin (Daunomycin). [206] Cardiomyopathy associated with doxorubicin therapy has been recognized since the early 1970s and has specific pathologic characteristics, including interstitial edema, cellular degeneration with irregular myofibrils, and myofibrillar dropout with dilation of the sarcoplasmic reticulum resulting in pale cells with cytoplasmic vacuolization. Mural thrombi may be seen within the heart.[207] Daunorubicin, an analog of doxorubicin, has a similar potential for causing myocardial damage. The incidence of anthracycline cardiotoxicity ranges between 0.4% and 9.0%, with a predicted mortality rate as high as 61% in some series. [208,209 and 210] Although acute changes have been described between 0 and 231 days (mean, 33 days) after the last injection, clinical and subclinical changes may not occur for many years after completion of therapy. [211,212] Theonset of symptomatic cardiac dysfunction may be subtle, with unexplained tachycardia, nonspecific cough, shortness of breath, a gallop rhythm, cardiomegaly, congestive heart failure, hepatomegaly, ankle edema and, frequently, pleural effusions. [213] Late dysrhythmiashave been reported 6 to 19 years after anthracycline therapy. [214] Patients with preexisting abnormalities seen on electrocardiography (ECG) may have an increased risk for cardiomyopathy. [215] Early-onset cardiotoxicity, occurring during therapy or within 1 year of completion of therapy, is the most significant risk factor for the development of late-onset cardiotoxicity (defined as occurring more than 1 year after completion of therapy). Unlike anthracycline cardiotoxicity occurring in children, late-onset clinically significant cardiotoxicity in adults is rare if not preceded by early toxicity.[215] Other risk factors for both acute and chronic anthracycline cardiotoxicity have been studied best in adults and include total cumulative anthracycline dose, dose schedule, size of individual dose, rate of infusion, gender, age at time of treatment, and concurrent exposure to other potential cardiotoxins. A recent pediatric study identified as risk factors for early cardiac toxicity in children an individual anthracycline dose exceeding 50 mg per $m^2$, a cumulative anthracycline dose exceeding 550 mg per $m^2$, black race, female gender, presence of trisomy 21, and treatment with amsacrine.[216] Infusion rate was not shown to be a risk factor for early toxicity in children. [217]

The risk factors for late toxicity in children are not as clearly defined and are based on data in adults. In adult studies, the incidence of symptomatic cardiomyopathy as it relates to cumulative anthracycline dose ranges from 1.5% at 350 mg per $m^2$ to 2.7% at 450 mg per $m^2$ and increases in a dose-dependent fashion from 6% at 550 mg per $m^2$ to 50% at 950 mg per $m^2$. [209,211,217,218] The schedule of drug administration dose and the rate of infusion may influence the incidence of cardiotoxicity. Limiting the peak plasma level may reduce damage to the cardiac tissue. Smaller, more frequent individual doses appear to produce less cardiotoxicity than do larger less frequent doses.[219,220 and 221] Giving anthracyclines by prolonged continuous infusions over 24 to 96 hours has been shown to lower the incidence of myocardial damage in refractory breast cancer patients, with the maximum protective effect at 96 hours.[222,223 and 224] Age, gender, and pregnancy also have roles in the development of late cardiotoxicity. Patients aged 70 years and older and very young children may be more susceptible to the cardiac effects of both doxorubicin and daunorubicin.[225,226] As compared with male patients, female patients have an increased risk of cardiac dysfunction after anthracycline therapy. [227,228] Although the reason for this gender disparity is unclear, Lipshultz et al. [228] speculated that it might reflect increased body fat in girls, with decreased clearance of anthracyclines and resulting increased exposure of non–adipose tissue (including the heart) to the drug. [228] Peripartum cardiomyopathy occurring years after completion of anthracycline therapy also has been reported.[227,229]

The combination of doxorubicin and radiotherapy to the chest is associated with an enhanced risk of cardiac damage. Radiation dosages as low as 1,260 cGy to the chest have been reported to potentiate the cardiotoxic effects of doxorubicin, even at anthracycline dose ranges considered to be safe. [209,215,230] Several other anticancer agents can potentiate the risk of doxorubicin-induced cardiotoxicity, including cyclophosphamide, dactinomycin, dacarbazine, and mitomycin C. [201,231,232 and 233]

In massive doses, alkylating agents by themselves may cause cardiomyopathy, as best documented with cyclophosphamide (120 to 240 mg per kg over 1 to 4 days), in conjunction with BMT.[234,235 and 236] This form of cardiotoxicity usually is reversible. High-dose cyclophosphamide has been reported to produce severe hemorrhagic cardiac necrosis.[237] The long-term implications of these acute problems for survivors is unclear. Other complications in the transplant setting include cardiomyopathy with or without associated pericarditis, pericarditis alone, congestive heart failure, and arrhythmias, including sinus tachycardia, atrial arrhythmias, bigeminal ventricular extrasystoles, and sinus bradycardia. A recent report suggested that the risk for cyclophosphamide cardiotoxicity in patients who have undergone BMT for aplastic anemia may be minimized by using a dose of 1.55 g per $m^2$ per day for 4 days, rather than the conventional schedule of 50 mg per $m^2$ per day for 4 days.[238] Twice-dailydosing regimens as compared with once-daily high-dose regimens also appear less toxic. [239]

Other anticancer agents have been reported to cause a variety of cardiotoxic events. Angina is an unusual complication after the use of 5-fluorouracil. [240] Mitoxantrone, an anthracine derivative but not an anthracycline, was developed in the hope that it might replace doxorubicin. Although very effective in acute myeloid leukemia, it does produce cardiotoxicity, and its effect is additive to previous myocardial injury. [241] Amsacrine (m-AMSA) is an acridine derivative with antileukemic activity and a potential for cardiotoxicity,[242] including ECG abnormalities, ventricular and atrial arrhythmias, congestive heart failure, and sudden death. Finally, busulfan, mitomycin C, vincristine, and VP-16 have all been associated with one or more cases of unusual cardiotoxic events. [206]

Direct or scattered irradiation to the mediastinum causes radiation-induced heart disease, which is mediated principally by vascular damage and fibrosis. The parietal pericardium is affected most commonly, resulting in fibrosis, constrictive pericarditis, effusion, and tamponade. [243,244,245 and 246] Myocardial damage is rare but more likely fatal and may include myocardial infarct and conduction system defects. Due to intimal proliferation of myofibroblasts and collagen and lipid accumulation resulting from irradiation to the heart, an increased relative risk for development of coronary artery disease and myocardial infarction has been observed in survivors of Hodgkin's disease and breast cancer after radiotherapy to the chest. [248,249,250 and 251] Risk factors for the development of coronary artery disease include age younger than 21 at time of treatment, larger daily fractions of radiation, a total dose exceeding 4,000 cGy, delivery of therapy to the midplane of the mediastinum, and lack of cardiac shielding. [252,253,254,255 and 256] Measurable cardiac dysfunction has been reported in patients undergoing spinal irradiation for treatment of CNS. [257] Vasoocclusive disease in vessels other than the coronary arteries—including the carotids, iliofemoral, vertebral, renal, and mesenteric arteries—has been reported after local radiotherapy.[258,259]

Detection and management of presymptomatic cardiac dysfunction in cancer patients require a high level of suspicion and close interaction with cardiology specialists. Because of the known long latency of cardiotoxicity after anthracyclines or irradiation in some cases, and because of the as-yet poorly defined late natural history of cardiovascular problems in patients treated as children, yearly examination for life is required in patients at risk. Generally, agreement about the best battery

of screening tests is not universal. Routine ECG, chest radiography, and cardiac enzyme analysis may demonstrate abnormalities but have not proven sensitive or predictive in the early detection of cardiomyopathy.[260,261 and 262] An association between prolongation of the QTc interval on routine ECG and increasing cumulative anthracycline dose (in excess of 300 mg per $m^2$) has been described in survivors of childhood cancer therapy but also is not proven to be predictive of later symptomatic cardiac deterioration.[263,264] The measurement of cardiac troponin T in the circulating blood has been identified as a marker of myocardiocyte damage and may prove useful as an indicator of acute anthracycline-induced cardiac inflammation.[265,266]

Subclinical myocardial damage evidenced by a decreased left ventricular ejection fraction may predict congestive heart failure, and the percentage of patients with abnormal ECG results and the degree of abnormality increase over time.[267,268 and 269] Multigated radionuclide angiography or echocardiography every 2 to 5 years is indicated for patients treated with 300 mg per $m^2$ or more of anthracyclines or with lower doses together with mediastinal irradiation. Whether the more conservative recommendations of the Cardiology Committee of the Children's Cancer Group—that electrocardiograms and echocardiograms be obtained at least every 2 to 3 years from patients who have received any anthracyclines[270] —will better detect clinically significant changes is unclear. Recent adaptations of echocardiographic measurements of cardiac function that have been developed, such as the stress velocity index, address some of the limitations of shortening fraction as a determinant of cardiac function. According to this index, 57% of 115 childhood leukemia survivors had evidence of cardiac abnormality 1 to 15 years after completion of therapy.[271] Increased after load due to reduced wall thickness was progressive in 71% of patients serially evaluated and was observed more commonly than was diminished contractility alone.[271] These sophisticated measurements may prove useful in long-term survivors. Multigated radionuclide angiography–based measurements of ejection fraction with exercise have been found by some investigators to add sensitivity to predictions about exercise tolerance.[272]

Percutaneous endomyocardial biopsy is the most direct and accurate measure of anthracycline cardiotoxicity and has been used in many centers for grading cardiac histology, predicting cardiac toxicity, and recommending therapeutic modifications in adult cancer patients and survivors.[273,274]

Prevention of cardiotoxicity by different dose schedules for anthracycline administration has been discussed. In addition, simultaneous use of cardioprotectants, such as ICRF-187,[275] and use of less cardiotoxic analogs of the anthracyclines are under investigation. This is especially important because historically, the long-term prognosis for patients with symptomatic, anthracycline-induced congestive heart failure has been suboptimal.[212,213] Despite aggressive medical management, the overall mortality in pediatric patients who develop symptomatic cardiomyopathy from cancer therapy may exceed 50%. Patients with asymptomatic cardiac dysfunction, including evidence of increased afterload, may benefit from afterload reduction[276]; a prospective trial of enalapril in pediatric cancer survivors with anthracycline toxicity is ongoing in the Pediatric Oncology Group.

## PULMONARY FUNCTION

Both the airways and the pulmonary interstitium are sites of significant late toxicity of anticancer therapy. In adults and adolescents in whom these problems have been sought and studied most extensively, pulmonary fibrosis with loss of lung volume, lung compliance, and diffusing capacity of carbon monoxide ($D_{LCO}$) in conjunction with pneumonitis most commonly is a result of pulmonary irradiation. Thus, these problems are seen most often in patients with thoracic malignancies, notably Hodgkin's disease and carcinoma of the lung. In such persons, asymptomatic radiographic findings or restrictive findings on pulmonary function testing consistent with fibrosis or pneumonitis have been reported in 30% to 100%.[277,278 and 279] These changes have been detected months to years after radiotherapy, most often in patients who suffered radiation pneumonitis as an acute toxicity.[280] Clinically apparent pneumonitis with cough, fever, or dyspnea occurs in only 5% to 15% of patients, however, and generally it does not develop except when more than 3,000 cGy in standard fractions has been delivered to more than 50% of the lung.[280,281 and 282] A prospective study examined pulmonary function at least 3 years after treatment of Hodgkin's disease using 44-Gy mantle irradiation.[279] None of 145 patients were symptomatic; only 30% of patients had forced vital capacities less than 80% of normal, and 7% had reduced $D_{LCO}$. A retrospective study from the same institution in patients all younger than 16 years at diagnosis and treated with low-dose involved-field radiotherapy (15 Gy) in combination with chemotherapy showed a similar percentage of asymptomatic abnormalities.[200] Recent data indicate that radiation-related pulmonary injuries likely are mediated by cytokine production—notably transforming growth factor-a, transforming growth factor-b, and fibroblast growth factor—that stimulates septal fibroblasts, increasing collagen production and resulting in pulmonary fibrosis.[283,284 and 285]

The incidence of radiation-induced late pulmonary toxicity has decreased dramatically in the last decade secondary to refined techniques in radiotherapy.[286,287,288 and 289] In a recently published study of patients with stage I and stage IIA Hodgkin's disease treated with irradiation alone, the late pulmonary effects observed were minimal.[288] Although vital capacity (VC), residual volume, forced expiratory volume in 1 second ($FEV_1$), $D_{LCO}$, and total lung capacity were decreased significantly at completion of radiotherapy as compared with pretreatment study results, all except $D_{LCO}$ returned nearly to normal within 1 year. The decrease in $D_{LCO}$ remained stable, but the forced expiratory flow rate at between 25% and 75% of VC showed a significant decline at 3 years after treatment as compared with results in baseline studies. Although age previously had been considered a risk factor for pulmonary toxicity in some studies, this new study was unable to identify any specific risk factors, including age, history of tobacco use, or preexisting lung disease.

On the basis of a few pediatric series, the mechanism for respiratory damage in young children appears to be different from that in adults or adolescents. In one study of 12 survivors of Wilms' tumor 7 to 14 years after treatment with bilateral pulmonary irradiation for metastatic disease,[289] several patients had dyspnea on exertion and radiographic evidence of interstitial and pleural thickening. Mean total lung volumes and $D_{LCO}$ were reduced to approximately 60% of predicted values in the face of normal “static elastic properties” of the lung after median total doses of approximately 2,000 cGy. In contrast to older children and adults in whom (as noted) irradiation for thoracic malignancy results in pulmonary fibrosis with loss of lung volume alone, these data were thought to be consistent with a proportionate interference with the growth of both the lung and the chest wall. Restrictive lung changes after lower doses of whole-lung irradiation (1,100 to 1,400 cGy) have been reported in several other studies of children with various malignancies.[290,291 and 292] Some investigators have suggested that children younger than 3 years at the time of therapy experience more chronic toxicity.[292]

Craniospinal irradiation for patients with malignant brain tumors also poses a significant risk for the development of late restrictive lung disease, although it is not accepted routinely as a pulmonary risk factor. A substantial number of brain tumor survivors treated with either craniospinal irradiation alone or irradiation combined with chemotherapeutic regimens (with or without lomustine) had evidence of restrictive lung disease on pulmonary function testing.[293] Obstructive changes also have been reported after conventional radiotherapy.[290] Obstructivelung disease was the chief problem in a large prospective series of patients with hematologic malignancy or aplastic anemia undergoing BMT. After 1,000 cGy TBI in a single fraction, 8% of patients had an $FEV_1$/ VC (a measure of obstructive lung disease) of less than 50% of normal at 3 years, and 29% had an $FEV_1$/ VC of less than 70% by that time. Unlike the transient acute restrictive changes observed in the same population, obstructive changes were not associated with a history of interstitial pneumonia nor were they associated with chronic GVHD.[294]

In addition to radiotherapy, a growing list of chemotherapeutic agents appears to be responsible for pulmonary disease in long-term survivors. Bleomycin toxicity is the prototype for chemotherapy-related lung injury. Although this problem has been reported in children,[295] clinically apparent bleomycin pneumonopathy is most frequent in adults, particularly those older than 70.[296,297 and 298] The chronic lung toxicity appears to result from persistence or progression of abnormalities developing within 3 months of therapy. Like the acute toxicity, it is dose-dependent beyond a threshold cumulative dose of 400 units and is exacerbated by concurrent or previous radiotherapy[296,299,300] or cyclophosphamide[298,301,302] or subsequent oxygen therapy.[303] At doses in excess of 400 units, 10% of patients experience fibrosis, and 35% to 55% suffer severe symptoms in the face of combinations of the foregoing factors.[300,304] At lower doses, fibrosis occurs sporadically in 5% of patients, with a 1% to 2% mortality rate. In some series, bleomycin toxicity was anticipated on the basis of $D_{LCO}$ abnormalities.[305]

Alkylating agents also are thought to cause chronic lung injury. As with bleomycin, carmustine pulmonary toxicity is dose-related. Although toxicity has been seen with as little as 800 mg per $m^2$,[306] doses higher than 1,500 mg per $m^2$ result in a 50% incidence of symptoms.[307] In a careful clinicopathologic review of children with brain tumors, O'Driscoll et al.[308] reported restrictive changes with lung fibrosis up to 17 years after treatment, the common feature of which was carmustine (100 mg per $m^2$ every 6 to 8 weeks for up to 2 years).[308] Four of the patients still alive at the time of study experienced shortness of breath and coughing; six showed a characteristic pattern of upper-zone fibrosis on chest radiography and CT scan; all had restrictive pulmonary function testing with vital capacities of 54% ± 19% of normal. Other contributing factors include the number of courses over which the drug has been given and a history of underlying lung disease, including asthma.[307] In two children, cyclophosphamide is thought to have caused delayed-onset pulmonary fibrosis with severe restrictive lung disease in association with marked reductions in the anteroposterior diameter of the chest.[309] This complication was postulated to have resulted from failure of lung growth during a period of rapid body growth. Melphalan[310] and busulfan[311] also are known to cause pulmonary fibrosis. Busulfan may be associated with a progressive, potentially fatal restrictive lung disease.

Other drugs associated with chronic pneumonitis and fibrosis are vinblastine[312] and methotrexate. Methotrexate toxicity, which probably occurs with an incidence

below 1%, generally has been associated with low-dose oral administration over more than 3 years.[313] Intravenous and, rarely, intrathecal[314] administration also may be responsible. The problem has been seen after cumulative methotrexate doses of as little as 40 mg to more than 4,500 mg and has been noted first at the beginning of maintenance methotrexate for treatment of ALL or after 18 years of low-dose therapy, as used in the management of psoriasis.[313]

After BMT, children seem to be at less risk than adults for significant late pulmonary dysfunction.[315,316,317 and 318] Explanations for this difference may include the fact that children tend to have less severe GVHD and their lungs are more resilient and thus may heal more quickly. The transplant preparatory regimens are similar regardless of age and therefore are not thought to be contributing factors to the differences observed. Both restrictive and obstructive pulmonary changes have been described in children after marrow transplantation, although the duration of time during which the reported abnormality persists after transplantation is varied. In one study of 17 children who had marrow transplantation for ALL or aplastic anemia, 7 of 12 with acute leukemia had significant decreases in lung volume 3 months after transplantation, which persisted in 3 of 4 studied 18 months after surgery. However, only 1 of 5 with aplastic anemia had reduced lung volume at 3 months. The patients with aplastic anemia had received preparatory regimens of cyclophosphamide only, whereas those with leukemia received cyclophosphamide with either TBI or busulfan.[317] In a subsequent study of patients with mixed neoplastic diagnoses, pulmonary function was maintained with only a modest, temporary drop in $D_{LCO}$ at 6 months after transplantation, with return to normal function by 15 months after surgery.[316] That patient group received a variety of preparatory regimens, including cyclophosphamide-TBI, cyclophosphamide-busulfan, cyclophosphamide-busulfan-etoposide, cyclophosphamide-etoposide-carboplatin, or TBI-melphalan. One difference between these two studies was the requirement for normal pre–marrow transplantation pulmonary function in patients in the second study.

Other factors contributing to chronic pulmonary toxicity include superimposed infection, underlying pneumonopathy (e.g., asthma), cigarette or respirator toxicity, and the effects of chronic pulmonary involvement by tumor or reaction to tumor. For example, a subset of patients with histiocytosis X (Langerhans' cell histiocytosis) will develop histiocytic pulmonary infiltrates or honeycombing with severe chronic restrictive lung disease unrelated to therapy or the presence of active tumor (see Chapter 26).[319]

Symptoms of pulmonary dysfunction, such as chronic cough (with or without fever) or dyspnea, should be sought on yearly follow-up, particularly in patients treated with thoracic irradiation, bleomycin, or carmustine; in patients who experienced acute pulmonary toxicity during therapy; and in patients with structural abnormalities of the thorax. All patients must understand the risks of smoking. Whether or how often to recommend pulmonary function tests or chest radiography in the long-term survivor in the absence of symptoms is not clear. Pulmonary function tests (including that of $D_{LCO}$) should be performed in patients with symptoms or in those who require general anesthesia for any reason. Because knowledge of baseline radiographs may be useful in managing intercurrent disease, chest radiographs should be obtained every 2 to 5 years, even in the absence of symptoms. Pulmonary function tests and possibly lung biopsy may be indicated if the chest radiographs suggest fibrosis.

The best approach to chronic pulmonary toxicity of anticancer therapy is preventive and includes careful monitoring of pulmonary function tests and chest radiographs before and during bleomycin or radiotherapy; respecting cumulative dosage restrictions on bleomycin administration; and limiting radiation dosage and port sizes.

## GASTROINTESTINAL FUNCTION

Fibrosis and enteritis are the most common pathologic abnormalities of the gastrointestinal tract in long-term survivors of cancer. These abnormalities can arise as late complications of irradiation to any site from the esophagus to the rectum[320,321,322,323 and 324] and have been associated with adhesions or stricture formation (sometimes with obstruction), with ulcers, and with malabsorption syndromes.[320] Their frequency depends on the radiation dosage delivered by external beam or by internal implants (as in patients with cervical or uterine carcinoma). The stomach and small intestine appear to be more radiation-sensitive than is the colon or rectum. Overall, the incidence of fibrosis after 4,000 to 5,000 cGy is 5%, and the incidence of fibrosis is as high as 36% after 6,000 cGy or more. Most complications of intestinal fibrosis arise within 5 years, but strictures have developed as long as 20 years after therapy.[320,324] Once they occur, radiation-induced gastrointestinal strictures may be progressive or recurrent. The incidence of clinically significant problems is enhanced by radiomimetic chemotherapy[321] or abdominal surgery.[320,321] These modalities by themselves can cause a similar array of problems.

Radiation also is a cause of chronic fibrosis of the liver, notably in patients treated with now-obsolete doses for Wilms' tumor or abdominal neuroblastoma. The degree of damage increases with the volume irradiated, prior partial hepatectomy, concomitant use of dactinomycin, the presence of large intra-abdominal masses compressing the liver or hepatic venous system and, possibly, younger age.[325,326 and 327] A trend, although statistically insignificant, also has developed toward increasing damage (both clinically appreciable and asymptomatic) with increasing dosages between 1,200 and 5,800 cGy. Radiation-induced hepatic fibrosis may develop in patients without a history of acute hepatopathy. In one series of 99 patients evaluated within 6 months of irradiation and again an average of 47 months after irradiation, 36 who were thought to have had normal liver function during the acute phase by physical examination, liver function testing, and liver scans with or without biopsy developed one or more abnormalities.[325]

Chemotherapy even in the absence of radiotherapy may be a cause of chronic hepatopathy. In several early prospective studies of patients given methotrexate for ALL or psoriasis, the incidence of biopsy-proven hepatic fibrosis was as high as 80% after 2.5 to 5.0 years of low-dose daily oral methotrexate.[328,329 and 330] With intermediate doses of intravenous methotrexate, the incidence of fibrosis has been less than 5%.[331] In general, and apparently in contrast to what is seen after radiotherapy, methotrexate-related hepatic fibrosis stabilizes or resolves after discontinuation of the drug. The contribution of other chemotherapeutic agents that cause acute hepatopathy (e.g., 6-mercaptopurine) to chronic liver disease has not been studied well. Cirrhosis has been documented in small numbers of survivors of stage 4S neuroblastoma with extensive hepatic tumor treated with a number of nonantimetabolite drugs or limited doses of radiation only (less than 1,000 cGy) or even after resolution of tumor in the absence of any therapy.[332]

Viral hepatitis, most often related to past transfusions, is another cause of chronic liver disease in long-term survivors.[333,334] In one retrospective series of 658 survivors of childhood cancer who had been treated before routine screening of blood products, 117 (17.8%) were seropositive for hepatitis C[333]; 35% of these also were positive for hepatitis B with or without delta virus. Eighty percent of the seropositive patients had been transfused, so that in 20%, other risk factors appeared to have been responsible. Of long-term survivors of BMT for pediatric leukemia diagnosed prior to 1991, as many as 35% are estimated to be seropositive for hepatitis B or C. In a recent series of patients who had survived for longer than 10 years after BMT for hematopoietic malignancy, hepatitis C was the major risk factor for late development of cirrhosis; of 16 patients with cirrhosis, 15 had disease attributable to hepatitis C.[335]

The true incidence of hepatic pathology undoubtedly is higher than that suggested by current numbers, however, because the presence of cirrhosis seldom is reflected by abnormal liver function tests or hepatomegaly, because hypertransaminasemia may be asymptomatic, and because liver biopsies or liver scans are not performed routinely after therapy. Miscellaneous late effects involving the gastrointestinal tract include postsurgical blind loop syndromes, iron overload (sometimes with secondary cirrhosis), secondary malignancies (discussed in the section Second Malignant Neoplasms), and complications of gastrointestinal tract or liver GVHD.[336] Radiation-induced or chemotherapy-related (in conservative or myeloablative doses) venoocclusive disease, which is usually fatal but sometimes transient, has become chronic in a few cases.[337]

Because detecting significant hepatitis or cirrhosis with attendant risks of liver failure or hepatic tumors may be impossible without liver biopsy, suggesting foolproof guidelines for long-term follow-up is difficult. For those patients who had acute hepatotoxicity during therapy and for patients treated with hepatectomy, methotrexate, or hepatic irradiation (or right-sided abdominal irradiation as sometimes is used in Wilms' tumor), the potential consequences of excessive alcohol intake are emphasized. In such patients, a chemistry screen, including transaminase and bilirubin level assessments every 2 to 5 years, has been found to be cost effective. If persistent, abnormalities are evaluated further in collaboration with a gastroenterologist. Centers for Disease Control and Prevention recommendations for hepatitis C screening include patients transfused or having undergone transplantation before 1992.[338] Given the trend toward lower doses of hepatic irradiation, shorter courses of oral methotrexate, and the infrequent development of clinically apparent end-stage liver disease with current anticancer regimens, liver scans or biopsies are not performed routinely.

Newer approaches to the treatment of gastrointestinal malignancy, including both administration of radiolabeled monoclonal antibodies for the therapy of hepatomas and intrahepatic arterial chemotherapy, have not yet been examined with respect to possible delayed effects.

## URINARY TRACT FUNCTION

Both the upper and lower urinary tracts are sites of late effects of anticancer therapy. The clinical presentation of chronic nephritis in this setting is the same as that in other settings and may include fatigue, anemia, nocturia, hyposthenuria, edema, abnormal urinary sediment, salt wasting, hyperuricemia with or without gout,

hypertension, and progressive renal failure. Intermittent or persistent proteinuria [339] or renovascular hypertension[340] may be an isolated finding or may evolve into chronic renal failure.

Radiation in doses exceeding 2,300 cGy given over 4 to 5 weeks is a well-defined cause of chronic nephritis, most commonly reported in patients with the following tumors: soft tissue sarcomas of the abdomen and pelvis; primary tumors of the kidney, adrenal, or gastrointestinal tract; and abdominal lymphomas. It may begin months to as long as 13 years after therapy and may occur de novo or after acute nephrotoxicity. [339,340 and 341] Although direct radiation nephrotoxicity is the usual cause, one report suggests that radiation may cause retroperitoneal fibrosis with ureteral obstruction. [342]

Dactinomycin,[343] anthracyclines,[346] and cisplatin[347,348] may enhance the nephrotoxic effects of radiation (earlier onset or lower threshold dose). Cisplatin, the nitrosoureas, and high-dose methotrexate therapy are well-known nephrotoxics by themselves. Cisplatin renal toxicity, which occurs in 50% to 75% of patients, appears to depend on the duration of treatment and the dose: It is uncommon at total doses lower than 50 mg per $m^2$ per course. Partial or full reversibility has been reported in some series,[349] although in several studies in which cisplatin was given as 20 mg per $m^2$ per day for 5 days every 3 weeks, a decrease in creatinine clearance of as much as 40% persisted at least 2 to 4 years.[350,351] These decreases may or may not be accompanied by parallel increases in serum creatinine levels. Long-term tubular defects (e.g., hypomagnesemia) also may be a problem.[350] Renal tubular damage manifested by hyperphosphaturia, glycosuria, and aminoaciduria followed by an inability to acidify the urine (known as *Fanconi's syndrome*) has been associated with ifosfamide therapy. The chronically low serum phosphorus and acidosis can result in renal rickets, with decreased linear growth and bony deformities, especially in prepubertal and pubertal children. [352,353] A report from the Intergroup Rhabdosarcoma Study, however, suggested that these abnormalities may resolve over time.[354] Factors that may encourage kidney failure include the following: nephrotoxic antimicrobial agents,[341] such as aminoglycosides, vancomycin, or amphotericin; the use of cyclosporine and related compounds as part of GVHD prophylaxis; inadequate alkalinization of the urine before methotrexate administration; ectopic kidneys, which may sustain inadvertent radiation damage; retroperitoneal radiation fibrosis with hydronephrosis; secondary urinary tract infections; and renovascular stenosis. Although nephrectomy, notably in children with Wilms' tumor, is not a problem, it may amplify any subsequent injury to the remaining kidney.

Cystitis, the development of which has been linked epidemiologically to several viruses, may be seen after radiotherapy or use of the chemotherapeutic agents cyclophosphamide and ifosfamide.[355,356,357,358 and 359] Radiation doses to the bladder at less than 4,000 cGy have resulted in a 5% incidence of hemorrhagic cystitis, a figure that is increased with distal urinary tract obstruction, infection, or the concurrent use of radiomimetic agents. Cyclophosphamide given by itself has been associated with an incidence of hemorrhagic cystitis of approximately 10% and, in some reports, as high as 40%. In an early report, chronic and irreversible cyclophosphamide-induced bladder fibrosis was seen at postmortem evaluation in 25% of children who received the drug. [355] A substantial risk exists whether given parenterally or by mouth. Whether the risk is dependent on duration of therapy or cumulative dose is controversial, with investigators reaching differing conclusions. In a study by Stillwell et al.,[356] of 100 patients(mean age, 43 years; range, 5 to 77 years) with hemorrhagic cystitis after cyclophosphamide, 78% had gross hematuria, 93% had microscopic hematuria, and 45% had irritative voiding symptoms. More than 50 patients experienced continued bladder symptoms (burning, urgency, incontinence, dysuria) 1 week to 1 year after discontinuing cyclophosphamide, with 16 having symptoms that lasted 2 to 8 years. In 20 patients, recurrent symptoms developed 3 months to 10 years after the initial event subsided. In this population, receiving cyclophosphamide intravenously reduced the median duration of therapy and cumulative dose associated with onset of cystitis. A small number of patients developed this complication after a single intravenous dose. Five patients studied developed transitional cell bladder cancer. Previous case reports have described the onset of hemorrhagic cystitis several decades after completion of therapy. [355,357] With ifosfamide, a cyclophosphamide analog, the incidence has been as high as 45%. [358] The use of adequate hydration, diuresis, and bladder protection with mesna has reduced the incidence of this therapy-related complication (see Chapter 10). Abnormal bladder function is seen also in children with pelvic rhabdomyosarcoma in whom radiation has been used as a means of avoiding exenterative surgery. From 27% to 100% of such children have experienced dribbling, nocturnal enuresis, and frequency, with higher incidences at higher radiation doses.[359,360] In some cases, this situation has been associated with hydronephrosis. More than one-third of patients who had pelvic rhabdomyosarcoma and have undergone total cystectomy without irradiation subsequently have experienced hematuria or bacteriuria. [360]

Adenovirus is a well-known cause of hemorrhagic cystitis and nephritis, particularly in patients after marrow transplantation. This complication, compounded by high-dose chemotherapeutic regimens containing cyclophosphamide, results in significant morbidity and mortality. Transplantation survivors who developed such infections may suffer from lasting bladder and renal compromise.[361]

Thrombotic thrombocytopenic purpura, a poorly understood multisystemic disease in which the kidneys are a primary target, has preceded institution of chemotherapy in adults with carcinomas.[362] The condition has been described after use of mithramycin [363] or the combination of cisplatin, bleomycin, and vinca alkaloids. [364] The closely related hemolytic uremic syndrome has been described in a high percentage of children 3.5 to 7.0 months after BMT. [365] Although these toxicities are acute and usually occur within 6 months of chemotherapy, they can lead to chronic renal failure and, therefore, are a potential problem in long-term survivors.

Other late effects related to the urinary tract include hyperammonemic encephalopathy [366] and hyperchloremic metabolic acidosis,[367] both of which have been described as rare complications of ureteral diversion. Neurogenic bladder with resultant incontinence has been reported in survivors of sacrococcygeal germ cell tumors.[368]

Monitoring long-term survivors at risk for urologic toxicity on the basis of their having received any of the foregoing therapies is straightforward. It should include the following precautionary measures: questioning patients both for the aforementioned signs and symptoms of chronic renal failure and for symptoms of hypertension or urinary tract infections; measurement of blood pressure, serum urea nitrogen, and creatinine levels; and urinalysis. Measurement of creatinine clearance or glomerular filtration, which may be more sensitive than simple urea nitrogen and creatinine measurements, can be implemented if the level of suspicion is particularly high. For patients with a history of tubular wasting, electrolyte levels (including calcium, phosphorus, and magnesium) should be checked intermittently. For patients also treated with irradiation, evaluation yearly or every other year may be indicated.

Hydration and diuresis with both hypertonic saline or mannitol and slower infusions may all reduce cisplatin-induced renal toxicity. Management of patients who have minimal hemorrhagic cystitis during therapy usually is supportive, with simple hydration for mild cystitis. For ongoing or severe disease, urologic evaluation is essential. Cystoscopy should be performed in all patients with a history of cyclophosphamide (or ifosfamide) use and gross or microscopic hematuria. Urine cytologic studies and fulguration of bleeding points, intravesical infusions of formalin, or ureteral diversion may be necessary.

## HEMATOLOGIC AND IMMUNOLOGIC FUNCTION

The long-term hematologic sequelae of anticancer therapy include compromised immune function and decreased bone marrow reserve. One of the best-defined alterations in immune function is the impaired humoral immunity that follows splenectomy. Splenectomy has been associated with overall decreases in serum levels of immunoglobulin M and immunoglobulin A[369] and with reductions in specific opsonins. In a literature review of data regarding 403 children who had Hodgkin's disease and had undergone splenectomy as part of a staging laparotomy, 32 (8%) developed fulminant infections, generally with encapsulated organisms [370]; 16 of these patients died. Patients were as long as 3 years from diagnosis at the time of their infection, a finding that confirms abundant evidence that the risk of sepsis persists. Splenic atrophy with similar consequences has been reported after splenic irradiation (approximately 4,000 cGy) of Hodgkin's disease and non-Hodgkin's lymphoma.[371] More recent experience suggests that pneumococcal vaccination and the use of prophylactic penicillin for indefinite periods diminishes that risk in survivors of Hodgkin's disease.[372]

Decreases in serum levels of immunoglobulin M and specific anti–*Haemophilus influenzae* capsular antigen also have been seen in patients with Hodgkin's disease as late as 7 years after therapy with MOPP.[373] This effect may be potentiated by total nodal irradiation although, in the absence of a splenic port, total nodal irradiation does not impair antibody production on a long-term basis. To what extent prior immunity for well-child vaccinations is abrogated in long-term survivors has not been tested rigorously for most anticancer regimens. After BMT, however, evidence indicates a progressive decline in antidiphtheria tetanus antibodies. [32]

Impairment of cell-mediated immunity, as indicated by decreased numbers of peripheral T cells and absolute lymphocyte counts, inversion of CD4/CD8 ratios, and depression of *in vitro* responsiveness to mitogens, also has followed total nodal irradiation in patients with Hodgkin's disease, [374] TBI,[375] and dose-intensive multiagent regimens (especially those containing more than 3.5 g cyclophosphamide per $m^2$).[376,377 and 378] Although absolute lymphocyte counts generally return to baseline within 3 to 6 months of therapy, incomplete T-cell reconstitution (especially CD4 lymphopenia) may persist for many years, especially in older children and adults and in patients experiencing GVHD. Prolonged $CD4^+$ depletion in excess of 30 years has been observed in Hodgkin's survivors after mediastinal irradiation, suggesting that the thymus is impaired irreversibly by local irradiation. [374,379] Significant abnormalities in the $CD8^+$ arm of the immune system also may persist even for years.[380,381] Some of these defects, which are greater in patients with more advanced disease, may reflect pretreatment abnormalities in part rather than resulting entirely from

therapy.

When irradiation has involved smaller nodal or marrow fields, long-term effects on the immune system have varied. In patients with gynecologic malignancy, no significant abnormality of either humoral or cellular immunity has been noted several years from diagnosis. [382] Conversely, 4 to 15 years after irradiation for localized laryngopharyngeal malignancy, depressed numbers of peripheral T cells and phytohemagglutinin reactivity have been noted. [383] Similar results have been reported after irradiation for carcinoma of the breast.[384]

In addition to producing its effect on lymphocytes, irradiation may compromise other bone marrow lineages in long-term survivors. Long-term bone marrow suppression after 3,000 cGy or more has been demonstrated by hypoplastic or aplastic aspirates and diminished uptake of radioisotopes with an affinity for active marrow,[385,386,387 and 388] by decreased granulocyte increments in response to endotoxins[389] and, less commonly, by peripheral cytopenia. The degree of marrow damage and, therefore, the clinical consequences depend on the dosage used and the volume irradiated: With 4,000 cGy of total nodal irradiation, peripheral granulocyte counts and bone marrow reserve can be impaired for as long as 7 years after therapy.[279,389] After 4,000 to 5,000 cGy in 4 to 6 weeks, complete recovery of marrow function may take more than 2 years.[387] After 850 to 1,000 cGy of single-dose TBI and marrow transplantation for various hematologic abnormalities, approximately 25% of patients have platelet counts of fewer than 100,000 per cubic millimeter even after 4 months. [390] Thrombopenia in this setting appears to correlate with the presence of GVHD. Whether this condition will be a problem for long-term survivors is not clear. Concomitant chemotherapy may increase the degree of radiation-induced marrow damage.[31,387]

The long-term effects of chemotherapy on bone marrow function have not been evaluated exhaustively despite the well-documented short-term effects. However, chemotherapy instituted as long as 3 years after irradiation in patients with Hodgkin's disease [391] or methotrexategiven as much as 18 months after craniospinal irradiation in patients with ALL [392] may result in long-term excessive myelosuppression. Age at the time of chemotherapy may have an important influence on the rate of recovery of helper T-cell function, because even young adults have fewer $CD4^{+}$ peripheral blood lymphocytes than do younger patients studied 6 months after chemotherapy.[393]

Monitoring long-term survivors for immunohematologic dysfunction is accomplished most conveniently by recording a detailed history by physical examination for signs and symptoms of recurrent infection, anemia, or bleeding diathesis. Peripheral cytopenia is useful but, as indicated, an abnormal complete blood count is not a sensitive marker of compromised bone marrow reserve. The approach to prevention or prophylactic management of immunocompromise has been pursued most aggressively in patients with Hodgkin's disease, in whom lifelong use of prophylactic antibiotics and single or serial injections of pneumococcal vaccine already have changed the natural history of postsplenectomy sepsis (see Chapter 41). In patients younger than 6 years at the time of splenectomy, the current recommendations are that *H. influenzae* vaccination be given as well. Recommendations for reimmunization of patients after BMT have been reviewed. [32] A trend in some institutions away from staging laparotomy, the use of partial rather than total splenectomy, and reduced reliance on larger doses of extensive radiation should eliminate some of these problems. How newer regimens of more intensive but shorter courses of chemotherapy will affect immune and marrow function is as yet unclear.

## SECOND MALIGNANT NEOPLASMS

People with a history of childhood cancer have been estimated to have 10 to 20 times the lifetime risk of a second malignant neoplasm (SMN) as compared with age-matched controls.[394] The incidence of SMN within the first 20 years after the initial diagnosis is on the order of 3% to 12%, [394,395] and SMN is the most common reason for death in long-term survivors, after the recurrence of the primary cancer. [396] Thus, in a recent follow-up of 13,610 5-year survivors of nonretinoblastoma childhood cancers, 488 SMNs were reported in 428 patients. [395] A small proportion of patients will go on to have two or more cancers after their initial diagnosis. However, these figures and the types of second malignancy differ according to the original diagnosis, patient age, specifics of therapy, and the presence of genetic conditions.

Childhood cancer survivors are at particularly high risk if their primary diagnosis was Hodgkin's disease, retinoblastoma, or the genetic form of Wilms' tumor; if they were treated with kilovoltage radiotherapy, alkylating agents, or epipodophyllotoxins; or if they have an underlying inherited susceptibility that also likely contributed to the development of their first cancer (e.g., von Recklinghausen's neurofibromatosis or Li-Fraumeni family cancer syndrome). Critical assessment of this evolving literature is complicated by the exclusion of noninvasive SMN, such as basal cell carcinomas and meningioma, from some series.

Acute nonlymphoblastic leukemia (ANLL), including myelodysplasias, is the most common hematopoietic SMN and has been reported in 10% to 20% of patients. [394,395] These figures are consistent with those from other single-institution or consortium studies. Based first on survivors of childhood and adolescent Hodgkin's disease, causation has been linked convincingly to mechlorethamine and cyclophosphamide, two alkylating agents commonly used in multiagent chemotherapeutic regimens (MOPP; cyclophosphamide, vincristine, procarbazine, and prednisone; and mechlorethamine, vincristine, procarbazine, and prednisone). [395,397,398,399,400,401,402 and 403] Within the pediatric population, adolescent girls may be at higher risk than are younger patients. [397] In contrast, ANLL after Adriamycin, bleomycin, vinblastine, and dacarbazine has been uncommon.[200,402,404] A contributory role for therapeutic doses of nodal radiotherapy in the development of secondary leukemia has been arguable, and data from some[399] but not all[397,400] series suggest that after irradiation alone, the risk of ANLL is slight and that MOPP alone carries the same risk as MOPP plus irradiation. The risk of secondary ANLL appears to plateau by 10 years from initial diagnosis. [394,405]

Alkylator-related secondary leukemias after Hodgkin's disease and other primary cancers have a number of characteristic features: a mean latency of 5 to 7 years (range, 3 months to 21 years), often after a myelodysplastic prodrome, and monosomy 5 or monosomy 7.[406] Secondary ANLL after a range of solid tumors and leukemia also has been ascribed to the epipodophyllotoxin etoposide (VP-16). [406,407,408,409,410 and 411] First reported in patients with T-cell ALL with an incidence of 3.8% at 6 years,[410] these secondary leukemias as a group have characteristics different from those that follow alkylating agents: They have a brief latency period (most are diagnosed less than 2 years from the initial diagnosis), demonstrate predominantly M4 or M5 morphology, and exhibit translocations within the MLL gene at chromosome band 11q23.[406]

However, these distinctions are not absolute. Some investigators have suggested a direct relationship between secondary ANLL and a cumulative dose of etoposide. However, results of a monitoring program from the Cancer Therapy Evaluation Program (CTEP) at the National Cancer Institute have shown no significant difference in 6-year incidence figures after doses ranging from less than 1.5 g per $m^2$ to more than 3 g per $m^2$.[412] Other risk factors include having received cancer treatment between 1980 and 1986 as compared with an earlier treatment era (possibly a surrogate for dose intensity), [394] intermittent administration of drug rather than continuous infusion, and concomitant use of other leukemogens.

Other chemotherapeutic agents may be leukemogenic: Platinum-based chemotherapy in the setting of adult ovarian carcinoma has been associated with an increased risk of ANLL.[413] Data from the Late Effects Study Group (LESG), Intergroup Rhabdomyosarcoma Study,[414] Denmark,[415] and National Surgical Adjuvant Breast Project[416] also have suggested a leukemogenic potential for doxorubicin, which (like etoposide) is a topoisomerase II inhibitor. Conversely, other studies specifically have not been able to implicate anthracyclines.[417] No patients who were included in the LESG, the Intergroup Rhabdomyosarcoma Study, or the National Surgical Adjuvant Breast Project databases and developed secondary ANLL had received doxorubicin alone, so that a clear link between ANLL and that drug is missing. Nonetheless, since 1994, CTEP has required investigators participating in National Cancer Institute–sponsored protocols to indicate the possibility of leukemogenic effects of anthracyclines on informed consent forms; in addition, all cases of SMN must be reported. Reports of ALL after treatment with doxorubicin and cisplatin without cyclophosphamide seem to support the CTEP decision.[418] An anecdotal report has associated chronic skin changes after subcutaneous infiltration of doxorubicin with the development of local melanoma.[419]

In contrast to leukemias, solid tumors have been attributed most commonly to radiotherapy, with two-thirds occurring within radiation ports. [394,395,403,414,420,421,422,423,424,425 and 426] The principal tumors have been breast carcinomas and bone and soft tissue sarcomas and carcinomas of the skin and thyroid, which appear with a median latency of 9.5 to more than 16.0 years and an actuarial incidence of 5.8% at 12 years. One report, in which 25 of 885 women with Hodgkin's disease developed invasive breast carcinoma and an additional patient developed multifocal carcinoma *in situ*, concluded that treatment with mantle radiotherapy before age 30 years is a risk factor for breast cancer.[420] The relative risk, which was more than 14-fold that of the general female population and higher than that of women similarly treated for other oncologic primary lesions, has been found to be especially high in patients who underwent irradiation between the ages of 10 and 15 years. Secondary breast cancer is not unique to Hodgkin's survivors and recently has been reported after whole-lung irradiation for treatment of pulmonary Wilms' tumor. [394] Data with regard to other primary tumor types from the LESG [403] and the CCSS[394] confirm the risk related to irradiation. Anthracycline exposure also may be a risk factor for breast cancer.[394] Adenocarcinoma of the breast has been attributed to radiation scattered from head and neck ports with doses to the breast as low as 16 mGy. [427] Sporadic cases suggest that angiosarcoma of the breast may follow irradiation. [428] The contribution, if any, of BRCA1 and BRCA2 genotypes to the risk for secondary

breast cancer of any pathologic type is the subject of ongoing investigation.

Thyroid cancer, which occurs after Hodgkin's disease at an incidence of approximately 18-fold that of the general population, [198,403] also may occur after low doses of radiation.

After cranial radiotherapy for ALL, in which the estimated dose delivered to the thyroid may be as much as 7.5% of the total, [202] a few cases of thyroid cancer and a similar number of adenomas have been reported.[429,430] Adenocarcinoma of the breast and salivary gland tumors also have been reported after low radiation exposures.[431] Adenocarcinomas of the colon and hepatocellular carcinoma have been reported in long-term Wilms' tumor survivors whose therapy had included now obsolete doses of radiation.[423,424]

Benign tumors, notably osteochondromas, also occur in irradiated fields and have been sought most carefully in survivors of Wilms' tumor. [29,36,425] Their malignant potential has not been defined. A recent multicenter series of more than 3,000 patients who had childhood ALL and had undergone BMT identified 25 secondary solid tumors.[426] Twenty-four of these (the majority of which were thyroid carcinomas and brain tumors) developed in patients who had received TBI, supporting concerns about the carcinogenicity of irradiation. Unlike the risk of secondary leukemias, the risk of nonhematologic malignancy may continue to increase over time, although recent reports with actual 20- to 30-year follow-up have shown that the rate of increase slows. [432,433]

The low incidence of SMNs in ALL patients treated with conventional therapy deserves separate mention because this group is the largest cohort of childhood cancer survivors. The risk has been estimated to be 62 in 100,000 patients per year, as compared with 280 in 100,000 for Hodgkin's disease survivors, [434] with a cumulative risk of 2.5% to 8.0% at 15 years from diagnosis.[417] As noted, a high-risk subset may be those children who have T-cell ALL and were treated with epipodophyllotoxins.[410] In the compilation of the LESG, the most common malignancies were other leukemias and non-Hodgkin's lymphoma.[434] An increased incidence of brain tumors (predominantly glial tumors, in contrast to the high incidence of secondary meningiomas after cns primaries) also was noted and has been the largest category of SMN after ALL in most other series, with a cumulative incidence of less than 2%. [394,417,426,435] High-risk factors appear to include therapy with at least 2,400 cGy of cranial irradiation or TBI and age younger than 5 years at the time of treatment. An unusually high incidence of 12.8% recently was reported of brain tumors among 52 survivors of ALL whose therapy, in addition to 1,800 cGy prophylactic cranial irradiation and intrathecal chemotherapy, included 6-mercaptopurine.[436] Of note was the presence of high erythrocyte concentrations of thioguanine nucleotide metabolites in the children who developed brain tumors. Supporting a contribution of 6-mercaptopurine to carcinogenesis is another recent clinicopharmacologic study from Scandinavia that reported an increased incidence of secondary ANLL in ALL survivors who had been treated with dose intensification of antimetabolites. [437] Although therapy contributes to causation of secondary brain tumors, the development of CNS and hematologic malignancies in the same patient might not be solely a function of therapy but may reflect a genetic connection between these two cancer types, similar to that between retinoblastoma and osteosarcoma (see Chapter 28 and Chapter 35).[434,438]

Carcinogenicity of other chemotherapy is not suggested strongly by available data. Indeed, dactinomycin, despite its potentiation of radiation with respect to other toxicities, appears to diminish the risk of radiation-associated second malignancies. [439] Such immunomodulators as cyclosporine or tacrolimus have been associated with secondary lymphoproliferative disease; in the respective settings of post–bone marrow or post–solid organ transplantation immunosuppression, the incidence is less than 1%[426,440] or approximately10%.[441] The multiple factors related to secondary malignancy in long-term survivors of BMT are reviewed elsewhere. [32,33,426]

Surgery plays a much more limited role in the development of second malignancies. Deserving of mention is the occurrence of benign and malignant colonic tumors at the anastomosis site of ureterosigmoidostomies at 500 times the expected rate.[442] Although surgical debulking of Wilms' tumor and soft tissue sarcomas has been supplanted by preoperative chemotherapy, many adults and some survivors of pediatric cancers with long-established ureterosigmoidostomies are at risk of developing colonic neoplasia. The average latency until the discovery of these cancers is 26 years. One observation is the association between splenectomy and the development of secondary acute myeloid leukemia in patients who have been treated with MOPP for Hodgkin's disease. [443] Other investigators have found this association to be of borderline or no significance. [433,444]

In assessing these statistics, and as pointed out by others,[414] the mean latency for solid tumors after radiotherapy is more than 15 years, and it is still too early to expect the common adult cancers to appear in survivors of childhood cancer, even if the incidence of these tumors ultimately will be increased. The need for lifelong surveillance for secondary malignancies is imperative. The relative merits of routine radiographic evaluation of bones or soft tissues encompassed by radiotherapy ports are discussed earlier in this chapter. The use of surrogate markers, such as sister chromatid exchanges, mutation frequency at the hypoxanthine phosphoribosyltransferase locus, or glutathione- *S*-transferase mutations in peripheral blood lymphocytes or buccal cells to predict the subsequent development of SMN is investigational.

## SURVEILLANCE

Recognition of delayed consequences of cancer therapy is one aspect of the study of late effects. One stumbling block to the application of this knowledge often is the inability to determine what therapy affected patients have received. Therefore, the first step in any evaluation is to have at hand an outline of such patients' medical history and especially a treatment summary. This can be part of affected patients' medical records and used whether survivors of pediatric cancer are seen in the setting of a dedicated late effects clinic among the growing number of transitional programs that combine pediatric and medical expertise [445] or, as is more likely, by medical oncologist, internist, family practitioner, nurse practitioner, or obstetrician-gynecologist. Particularly before long-term survivors of childhood cancer “graduate” to the care of nonpediatric oncologists, this treatment record and possible long-term problems should be reviewed with involved families and, in the case of adolescents, with patients. Correspondence between a pediatric oncologist and subsequent caregivers should address these same issues. A typical interim history designed to focus on significant medical problems and problems of psychosocial readjustment, school and job performance, and insurance is summarized in Table 49-3. Table 49-1 summarizes the late effects that are discussed in this chapter and that should be sought in patients who have received particular forms of chemotherapy, irradiation, or surgery. A complete physical examination in which one specifically looks for these late effects is routine.

As noted, because of the delayed onset or potentially progressive nature of some of these problems, such evaluations often bear repetition yearly or every other year. In addition, the same preventive health considerations directed at the population at large are warranted at least to the same degree in long-term survivors of childhood cancer. These include avoidance of smoking and excessive alcohol consumption, monthly self-examination of breasts or testes, and other cancer-related screening checks. Although long-term survivors of childhood cancer generally are thought to be cured of their primary malignancy, the possibility of late recurrences needs to be kept in mind. The greatest cause of death beyond 5 years from diagnosis remains recurrent tumor. [396,446]

Recommendations for laboratory tests are individualized according to problems anticipated by physicians, based on patients' disease and, especially, on therapeutic history. These recommendations are summarized in Table 49-2. Many of the late effects of therapy are laboratory phenomena with unknown clinical consequences and for which interventional strategies have not been defined. Surveillance for late effects is an evolving issue.

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# EDUCATIONAL ISSUES FOR CHILDREN WITH CANCER

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## INTRODUCTION

Each year in the United States, approximately 8,000 new cases of cancer occur in children younger than 15 years. [1] As medical treatment has advanced over the last 30 years, childhood cancer has increasingly come to be viewed as a life-threatening chronic illness rather than a terminal illness. One in every 1,000 U.S. adults between the ages of 20 and 29 years is now a survivor of childhood cancer. [2] As the rate of survival increases, the quality of life of survivors takes on added importance. In his book, *The Truly Cured Child,* Van Eys[3] challenged professionals working with children with cancer to reconsider the definition of *cure*. He defined a truly cured child as one with "social, mental, and physical well-being" and a "child who becomes an adult able to live to the full extent of his talents." [3] Because education is crucial to the realization of a child's full potential, a partnership between health care professionals and school personnel is important to patients' and survivors' quality of life.[4,5 and 6] Communication among all professionals who participate in children's care, including school personnel, is not a luxury but an essential element in the total care of children. [7]

Children with cancer present a unique set of challenges to any school system. Some problems, such as absences and resulting poor performance, may be of short duration, whereas others may be long-term developmental problems that require ongoing assessment. [8,9] This chapter approaches school reentry and intervention as the ongoing processes they must be. Proactive, preventive assessment and intervention must be made an integral part of children's treatment and the long-term follow-up process.[10]

## THE IMPORTANCE OF SCHOOL REENTRY INTERVENTION

School is the work of childhood. It presents each child with a daily opportunity to feel productive, master the environment, learn social skills, and receive peer support. As Maul-Mellott and Adams[11] stated in *Childhood Cancer: A Nursing Overview,* "The regular achievement and long-range planning required in the school setting validate the future for children. The acquisition of skills and mastery of complex principles are aimed toward preparing the child for the larger arena of life. In this way, participation in school reinforces the fact of the future for all children. It affirms the probability of living to use the skills gained." Thus, children with cancer who are denied school participation are, in effect, denied an important opportunity to engage in age-appropriate, goal-oriented behavior. [12] Such children may acquire a sense of learned helplessness that reinforces feelings of hopelessness and despair, obstructing their ability to cope with their illness and the rehabilitation process. [12]

Many authors have advised that children and adolescents with cancer return to normal activities such as school as soon as medically possible. [6,13,14,15,16,17,18 and 19] In this manner, at least a part of their lives is returned to normalcy in the midst of their illness and medical treatment. Van Eys [3] wrote, "A child's development continues when he has cancer . . . But the environment must be conducive to normal development. That environment is not just the one created by the parents at home, but the sum total of all experiences that the child has during his illness. The child must be allowed normal development during abnormal circumstances." Thus, school reentry becomes a part of the treatment process, and teachers and school system become a part of the treatment team.

Problems that sometimes create a barrier to school reentry include a patient's anxiety about peer teasing and rejection because of the visible effects of treatment; continued school absences; parents' reticence to allow such a child to return to school; a child's school phobia or separation anxiety; a teacher's overindulgence or unrealistic expectations about a child's abilities; a child's illness-related disabilities (e.g., fatigue, pain); and the need for special services or classroom accommodations.[12,15,20,21 and 22] However, children and adolescents often find that most of the social and emotional support they need for the return to school comes from classmates who have received education about an affected student's illness and treatment. [23]

Research conducted over the last 20 years on the problems of school reentry for children with cancer and other chronic illnesses indicates that intervention reduces problems and increases the likelihood of successful reentry. Katz et al. [13] studied the psychological and social functioning of children with cancer using a control and intervention group. The components of the intervention were (a) preparatory activities, including parent-child counseling and phone communication with school personnel to alert them to children's general needs for special services; (b) face-to-face conferences with school personnel about specific needs and reentry presentation; (c) actual classroom presentation, giving a general description of affected children's medical and hospital experiences; and (d) follow-up. The parents of children in the intervention group reported fewer behavior problems than did the parents of children in the control group. Children in the intervention group were also less anxious, less depressed, and had greater social competence after returning to school. Patients, parents, and teachers perceived the intervention as successful.

Katz et al.[14] later described a similar intervention with the addition of a designated school liaison and reported that teachers, parents, and children perceived the intervention as beneficial. The teachers reported that they and the classmates of children with cancer gained knowledge as a result of the intervention. They also reported good acceptance of such patients by peers as another positive impact of the intervention.

Ross and Scarvalone[24] described an intervention program for school personnel using a seminar format. The seminar offered general information about childhood cancer, treatment, and side effects; information about the psychosocial aspects of cancer treatment and ways in which school personnel can be helpful; a tour of the hospital; and small group discussions. Evaluation of the program indicated that the school personnel who were given information about affected children's disease, treatment, and related psychosocial issues felt more confident, were able to answer the questions of patients and classmates, could deal more effectively with parents, and could treat patients more as normal students.

Rynard et al.[25] reported the results of year-end teacher and parent evaluations of a school support program. The basic components of the program were (a) discussion with patient and parent and phone contact with school personnel to explain services; (b) provision of information to the school, including a film for classroom use, a teacher's manual about childhood cancer, a disease information sheet, and an individual medical summary for the child; (c) a meeting with school personnel, peers, and the child to help school personnel to anticipate the needs of patient and school community; (d) follow-up with the child and school personnel; and (e) an annual workshop for teachers, parents, and health professionals to provide additional information. Parents and teachers viewed the program as "highly useful."[25] The teachers found the school conference to be the most important component. Parents also rated provision of information to the school as very important. The results of this study strongly support the importance of links between school, hospital, and home. [25]

Benner and Marlow[26] described an intervention for first-, second-, and third-graders who had a classmate with cancer. The 30-minute presentation provided general information about childhood cancer, treatment, side effects, and the emotional aspects of cancer. After the presentation, the classmates showed increased knowledge of childhood cancer and an increased desire to interact with the child with cancer.

Finally, Varni et al.[27] went beyond the usual school reentry intervention. Their intervention was designed to improve the social competence of children with chronic illness, thereby facilitating positive social interaction with teacher and classmates. The results of this study suggested that social skills training may add to the benefits offered by the standard school reintegration intervention. Specifically, the group that received social skills training in addition to the standard intervention evidenced a

significant reduction in behavior problems, a significant increase in classmate and teacher social support, and a significant increase in social competence after 9 months.

## PHASES OF SCHOOL REENTRY

As a framework for our discussion of school reentry, we use a model of school reentry first described by Madan-Swain et al. [28] We integrate it with a stratification of student disabilities that can be used at reentry and throughout affected children's scholastic careers. This chapter also addresses school intervention for children with cancer, including how to obtain special education services and special classroom accommodations based on federal legislation.

According to Madan-Swain et al.,[28] school reentry occurs in three phases: phase 1, initial hospitalization and plans for reentry; phase 2, contact and education of school personnel; and phase 3, follow-up contact.

### Phase 1: Initial Hospitalization and Plans for Reentry

The process of school reentry should begin shortly after the diagnosis of cancer. Certain principal considerations are included in this phase. [13,26,29]

#### *Identification of a Hospital-School Liaison*

As early as possible, an affected child's physician or treatment team should obtain parental consent to assign a school liaison. The liaison should be a professional who has a background in education and can work with parents as an advocate for the child and serve as a bridge between the hospital and school personnel. [19] This professional should contact the child's school to discuss the child's diagnosis and initial absence from school. The liaison also may use this opportunity to discuss with school personnel any pertinent premorbid history, such as scholastic achievement, peer acceptance, and general social adjustment in the school environment. The school liaison should ask about the parents' history of supporting the child's achievement in school and about their cooperation and their attitude toward school personnel.[10]

Such information is helpful not only for anticipating school reentry needs but for understanding a child's learning style or possible learning disabilities and foreseeing what assistance such a child may need in understanding the diagnosis and treatment plan. Generally, children who have a history of premorbid learning or adjustment problems are at greater risk of difficult school reentry. [12,15,20,22,30]

#### *Physician Emphasis on the Importance of Returning to School*

Early in the treatment process, involved physicians should discuss the importance of children's returning to school and to other normal activities. The return to school should be discussed in terms of *when*, not *whether*. Parents who see the return to school as a normal expectation and part of the treatment plan are more likely to feel comfortable with the prospect of school reentry and to comply with the plan. [19] Although many parents and patients are eager to discuss and plan school reentry, some parents are more reticent about sending their child back to school because of anxiety about infection, potential peer rejection, or ridicule. Some parents also become emotionally enmeshed with their child during illness, and both parent and child experience separation anxiety. [21] School phobia also can begin at this time.

At least one study indicated some predictors that may be evident at this time, including avoidance of the topic of school and passive or active resistance to participation in alternative school services, such as home-bound or hospital-bound instruction. [21] These issues necessitate ongoing communication among parent, child, and physician about the continuation of education and school reentry. This communication will provide physicians an opportunity to gauge children's and parents' adjustment and compliance; will provide information to reassure anxious parents; and will arrange for the participation of other professionals, such as a psychologist or social worker, whose help may be needed. It will also give the patient and parents an opportunity to ask questions about any concerns they have about school reentry.

#### *Arrangements for Home-Bound or Hospital-Bound Instruction*

It is very important that children have some type of alternative educational services while they are unable to attend school. Although important for every child, ongoing instruction and learning are most significant for children who are beginning their education and are building foundation skills in mathematics and reading, for children with a history of learning disability, and for older adolescents who are near graduation. [10,15,31,32] An emphasis on continuation of school even while in the hospital or at home for extended periods reassures affected children about an expectation for a future. [33] Also, home-bound or hospital-bound education serves to decrease anxiety and hesitation about school reentry. [33]

Several options are available for the continuation of children's education during hospitalization or confinement at home because of immunosuppression. If such children are at home or are hospitalized near their home, their school is responsible for providing a home-bound teacher. If such children are hospitalized at such a distance from the home community that such provision is not possible, the school district in which the hospital is located may be responsible for providing education for the child. The hospital may have a school program that usually will communicate with the home school so that books and assignments can be sent to the teacher of a hospital-bound or home-bound student.[6,11,34] Such an arrangement will help to keep affected children in touch with what their classmates are doing.

School reentry also will be easier if such children know that they have been doing the same work from the same books as that performed by his peers at home. Some school systems now lend laptop computers to students who are hospitalized away from home so that they can use e-mail to send assignments and maintain communication with classmates. Other technologies that may be available include video teleconferencing with a child's classroom and the use of computer programs and the Internet to supplement educational materials. [10]

Teachers of hospital- or home-bound students probably will meet with affected children for two to four sessions per week, so some self-discipline on the part of these children and support from the parents are needed if such children are to keep up with assignments. Basic skills development is of the utmost importance during the hospital-bound or home-bound educational experience for younger children. At this age, building foundation skills in mathematics and reading is vital. [11,15,34] The subject matter probably will have to be prioritized for such students and for adolescents who have several subjects and teachers.

During this time, affected students probably will not be able to complete every assignment for every class. Also, some classes, such as drama, art, and the like, cannot be taught in the home or hospital setting; adolescents must be excused from them or be given alternative assignments. [10]

The school liaison should be aware of available hospital-bound and home-bound services. This liaison should work with affected parents and their children's school system to find the most appropriate method for continuing their children's education and to design a plan whereby such patients receive appropriate credit for work completed. The liaison can ensure the availability of a plan for dealing with subjects or classes that patients may not be able to undertake at all. Finally, it should be emphasized that home-bound and hospital-bound educational services are a temporary measure, with the ultimate goal being school reentry. [4,35]

#### *Providing Information to Classmates about a Child's Illness*

With the parent's permission, the school liaison can work with the teacher, counselor, or both to provide appropriate information to an affected child's classmates about the diagnosis and the anticipated length of absence. The liaison also can provide written materials, such as *Helping Schools Cope with Childhood Cancer: Current Facts and Creative Solutions*, authored by Chambers et al.,[36] and *Cancervive Teacher's Guide for Kids with Cancer,* written by Nessim and Katz.[32] These booklets can provide the teacher with some direction about how to talk with classmates and answer their questions.

Also important is that classmates be encouraged to communicate with children in the hospital or at home; this can be accomplished through e-mail, cards, phone calls, audiotaped or videotaped messages, hand-drawn posters and, when possible, personal visits. Keeping in close contact with classmates will give affected children a sense that they remain a part of the classroom, will help connect the hospital and school, will alleviate fears that such students will be forgotten by peers, and will reduce anxiety about peer rejection on reentry. [15,37]

### *Assessment of Level of Disability*

In the section Stratification of Disability Levels, we present a stratification of disability levels based on premorbid disabilities and chronic illness- and treatment-related disabilities. The level of disability should be assessed many times over a child's scholastic career, and changes in level should be expected. In phase 1, information should be gathered about affected children's premorbid functioning. Their school history and any premorbid history of learning or physical disability will be the first pieces of information to be considered in determining level of disability.

The next information to be considered is the presence of any chronic illness- or treatment-related disability. Such diagnoses as brain tumor and acute lymphocytic leukemia (ALL) for which central nervous system–directed therapies are used are associated most often with chronic illness–related disabilities. With regard to brain tumors, disabilities may be caused by the tumor itself or by the effects of surgical resection.[38] In the case of slow-growing tumors, disabilities that appeared before the diagnosis of cancer may have been caused by the tumor. Hence, a learning disability, cognitive deficit, or delay that was in evidence prior to diagnosis may be disease-related. In some cases, affected children's cognitive or academic functioning may improve after tumor resection and recovery from surgery. Children with other malignancies may have impairments such as limb amputation or visual impairment due to enucleation of one or both eyes. Chronic illness– or treatment-related disability will have the greatest effect on children's disability rating over time, as the long-term effects of treatment on learning emerge.

Also, during this initial phase, patients may begin serial testing and assessment of their neuropsychological functioning. This monitoring is most mportant for children who receive central nervous system–directed therapies, such as those who have ALL or brain tumors.[38] Repeated assessment will be essential for the detection of emerging treatment-related disabilities that may not be seen for several years after treatment.[8]

## Phase 2: Contact and Education of School Personnel

In phase 2, affected children actually will go back to school, if possible, and a classroom presentation will assist with school reentry. Children and adolescents usually want to return to school.[19] At school, they can be students, not patients, and school can provide a refuge of normalcy.[7] Furthermore, research has shown that the perception of social support from parents, teachers, and classmates is a predictor of positive psychological and social adjustment in children with chronic physical disorders.[39,40] In planning for school reentry, the different perspectives, expectations, and needs of all the participants must be considered. Certain concerns should be explored.

### *Child and Adolescent*

Planning for school reentry always should begin with an interview with affected children. As they consider their return to school, children and adolescents most often express concern or worry about peer rejection or ridicule because of hair loss or other change in appearance; falling behind academically or having to repeat a grade; and relating to teachers and other school staff.[12,15,20,21] and [22]

These problems are intensified for adolescents. Academic pressures are greater as adolescents move closer to graduation, so that the fear of falling behind or not graduating with one's class is greater. Adolescents also must interact with several teachers, each with their own perspective on the subject illness and how best to treat returning students. Secondary schools also tend to be larger and less personal than elementary schools.[41] Peer and social relationships are more complex at this age, as adolescents usually are more independent of parents and spend more time with peers.[41]

Children or adolescents also may be concerned about potentially stigmatizing situations, such as nausea, extreme fatigue, or frequent need to use the restroom during classes. Children with physical impairments may be concerned about being knocked down while navigating a crowded hallway or stairway.

Prior to the interview with affected children, it is important to gather as much information as possible about their current level of disability and their premorbid school adjustment and level of achievement. Children and adolescents who disliked school or were poor achievers before their illness may have more difficulty with school reentry.[10,37] Figure 50-1 is a form that may be used not only to gather information about affected children but to guide the interview with them and their parents and to record the information gathered.

**FIGURE 50-1.** School reentry checklist (**A** and **B**). IEP, individualized education program.

#### Child's Fears and Concerns about Returning to School

Although common concerns usually are expressed by children and adolescents, all children should be given the opportunity to express their individual concerns.[17] Discussion should include any previous problems affected patients may have had in school and how they may be related to fears or concerns they have now.

#### Services or Support Available to Assist Reentry

After fears and concerns are discussed, information should be provided about services available to assist reentry. If affected children live a fairly long distance from the hospital, telephone consultation with the teachers or guidance counselor and provision of appropriate written materials may be all that can be done. At this time, discussion should address any need for special education or special classroom accommodations and the federal laws that mandate the provision of these services to children with disabilities (discussed at greater length in the section Federal Laws Protecting the Educational Rights of Children with Disabilities ). Information about these services will assist in alleviating some fears and concerns of patients and parents.

Associating the need for special education services with the impact of the disease process and treatment, and identifying new deficits or weaknesses as “acquired,” may help returning patients to avoid perceptions that the services or the difficulties reflect on them personally. Reframing the condition as a side effect, as something the disease or treatment has done to them, may help to protect affected children's sense of self-worth. They may be less prone to see placement as a punishment for something they have done or failed to do. As a part of such reframing, the special education services can be identified as one more way for such children to fight back against the disease process.

#### Presentation about Illness and Treatment to Classmates or Teachers

Elementary-school children usually want someone from the hospital to go to their school. This author has seen the enormous relief that the prospect of a classroom presentation can bring to many children. The case is less well defined with middle-school and high-school students, for whom the need not to be different is paramount. Adolescents sometimes wish to tell only teachers and a close group of friends and to have the information disseminated to others through them. It is important to respect such patients' wishes in this regard, but equally important is to ensure that they are fully aware of available services and support and how these

services can benefit them.

Providing children with examples of what can be said or how situations have been handled by other children may ease some of their uncertainty or concerns. An example speech that refers to the *strength*, *courage*, and *determination* children show during their battle with cancer may also bolster their self-esteem.

**Topics That May Be Discussed with Teacher or Classmates**

If affected patients have agreed to a classroom or teacher presentation, the exact content of the presentation should be reviewed. *Always* ensure that such children are fully aware of, and agree to, everything that will be covered in the presentation. For example, some children may or may not want to discuss their central line. At this time, also, affected children should be assisted in anticipating some of the questions they are likely to encounter.

**Being Present and Participating in the Discussion with Classmates and Teacher**

In the authors' experience, most children and adolescents want to be present during the presentation and may wish to participate as an “expert” on their illness. Such children should be helped to formulate some “stock” answers to difficult questions at this time.

***Parents***

Parents have many concerns about their children's school reentry. The attitudes of parents range from thankfulness that such children can return to the normalcy of school to feeling that they do not want them to suffer more by being forced back to school.[43] Parents may be overprotective and feel that their children are too vulnerable to go out into the world.[30] Parents and children also may develop a mutual separation anxiety that can lead to school phobia in such children and can cause parents to refuse to allow school reentry.[21] In a survey by Charlton et al.,[34] parents of children who had solid tumors and were returning to school listed the best and worst things about school reentry: In the “best” category were a return to normal, seeing their child happy and reuniting with friends, and ensuring that the child did not get too far behind in work. On the “worst” list were worries about the child's inability to cope with school, possibility of physical injury, loss of hair, teasing by peers, and being behind in school work. These parents also reported that they had received discouraging opinions about school reentry from others in the family, such as grandparents, who may have had outmoded ideas about childhood cancer.

Affected parents' level of anxiety or comfort about school reentry and return to other normal activities definitely influences how children respond [12,20,34] and, in fact, can be crucial to school reentry success.[30] Those parents whohad little premorbid school participation may not believe that school reentry is very important and may not have skills necessary to help their children.[12] These observations reinforce the importance of communication among all professionals working with children with cancer.

Information received by the school liaison about the parents' premorbid school involvement should be shared. Everyone working with returning children should understand parents' attitudes toward school reentry and scholastic achievement in general and should work in a proactive manner to assist the parents in recognizing the positive aspects of school reentry while providing reassurance and information to decrease anxiety about problems such children may encounter. Any professional who detects a problem in this area should alert other professionals working with the family to ensure that the family receives the needed support, such as referral to a psychologist or social worker in the community or at the hospital.

For parents of children who have some illness- or treatment-related impairment, the prospect of school reentry may be especially intimidating. Such parents worry about teasing and the possibility of injury to their children and also must navigate through the school system's bureaucracy to get special services (special education, classroom accommodations, or both) needed by returning children. It is essential that such parents—and all parents of children with cancer—have access to information about available services, how they are accessed, what federal laws mandate that schools provide these services, and how to become an effective and assertive advocate for their children. Older adolescents also should have this knowledge and should become advocates for themselves. The school liaison should provide this information and written materials to parents to help them to understand this process better. The school liaison also should discuss with affected parents the specific needs of their children and should attend, with the parents, any important school meetings about such children. If the school liaison cannot attend such meetings because of distance, the possibility of a conference call during the meeting should be explored. Empowering parents and adolescents in this way can help them to regain a sense of control and a more positive outlook about school reentry.[42]

***Teachers***

The low prevalence of childhood cancer means that for most teachers and other school personnel, having a child with cancer in the classroom is a new experience.[43,44] Peckham[45] reported that in a school district of approximately 6,000, only seven or eight students will be patients or long-term survivors of cancer. The teacher plays a crucial role in adjustment to school reentry by influencing the tone of the classroom and helping classmates to understand returning children's physical changes and limitations.[46] It is, therefore, vital that affected teachers have a full understanding of such children's diagnosis and treatment and have access, through school liaisons or parents, to any other pertinent medical information.

Stevens[47] discussed several issues that teachers must confront when children with cancer are in the classroom. Emotionally, teachers must deal with their personal feelings about such children and the children's diagnosis of a life-threatening illness. They may experience grief, as do the friends and family of such children, and they may distance themselves emotionally.[45] They often want to provide emotional support for these children but feel unprepared to do so. They turn to other teachers and family members for support.[23]

Teachers experience anxiety about their lack of knowledge of childhood cancer, what their expectations of affected children's performance should be, and what medical problems might arise in the classroom and how to deal with them.[4,16] Such teachers want and need this information but feel uncomfortable about asking parents for it directly for fear of exacerbating their sadness about their children's illnesses. [4,23,24,30,46] This lack of information may lead to teachers' overprotecting such children and treating them as favorites because of feelings of pity.[4] Under these circumstances, returning students are not challenged to live up to their potential.

Conversely, involved teachers may lack information about the true limitations of such children and may have unrealistic expectations that lead to frustration and discouragement.[30] If such teachers were familiar with returning children before the advent of their illness, their expectations may not take into account changes that such children have undergone. When several teachers are involved, as in the case of adolescents, this problem is intensified.

Teachers may feel uncertain and unprepared to answer questions from classmates about returning children's illness and prognosis. Also, teachers may feel some conflict about the attention needed by children with cancer and may fear a conflict with the needs of the other children in the class.

The one factor that can prevent or alleviate these problems and fears is *communication*. Several studies have shown that teachers feel the need for more communication with affected children's medical team. Such teachers seek the provision of more medical information or information about affected children's functional level and performance expectations.[15,16,20,23,24,34] According to the previously mentioned study by Rynard et al.,[25] teachers rated the importance of a school conference very highly.

The school liaison should, as mentioned, communicate with returning children's teachers and other school personnel shortly after diagnosis of such children's illness to discuss the illness and the course of treatment and should provide some direction about how to talk with classmates and answer their questions.

Once the date of school reentry is known, the school liaison can meet with (or telephone) the teacher, other school personnel, and parents to make specific plans and to discuss expectations for performance, changes in level of ability and level of physical activity, and any accommodations or special services returning children may need. Such children's teachers should know what skills have been learned while working with the home-based or hospital teacher and the level at which such children will be reentering school. Teachers and school personnel also need to know what, if any, medical problems may arise at school, what to do or whom to call, and what medications affected children may need to take. Also important is information about infectious diseases; teachers should inform involved parents as soon as possible about any possible cases of influenza or cases of or exposure to chickenpox.

### *Classmates and Peers*

Peer socialization is a very important part of children's lives, and peers are a vital group in assisting school reentry. [4] This is especially true for adolescents. They feel a need for independence from parents and for strong peer relationships. [22,33] Maintenance of good peer relationships throughout treatment can assist in making affected children feel “normal” and “well” [48]; however, peer rejection is a paramount fear of both children and adolescents at the time of school reentry.

When children are absent from school for a period for any reason, peers will have questions about where they are and what is happening to them. If such children have cancer, the news can spread quickly, but inaccurate information also may be spread. [49] Classmates may overhear such inaccurate information from parents and teachers or may fabricate an explanation when their questions are not answered. Myths about cancer, such as its being contagious, also abound, even among older adolescents and adults, and can lead to affected patients' isolation from peers. Sometimes, for older adolescents, such misinformation can include the association between cancer and acquired immunodeficiency syndrome.

Teasing also is an issue. Some teasing is a normal part of the school experience, but sometimes children with cancer are targeted because of their perceived vulnerability or fragility or because of the visible signs of their disease, such as loss of hair. [20] Although children can be cruel, some tease because they do not know what to say or how to act around children with cancer. They may wait to get from such children cues about how to behave, but they do realize at some point that these children are the “same persons.” [20,29] Adolescent patients sometimes have been put in the position of having to comfort and support their friends instead of the opposite. [20] Peers can become overly nice or doting, and this behavior may be perceived just as negatively as teasing, especially by adolescent patients. [33]

Good peer education is an essential part of any school reentry plan. Classmates and peers need clear, accurate information presented at an age-appropriate level about returning patients' diagnosis; treatment; side effects of treatment, including any transient or chronic impairments (especially those that are visible); the course of treatment, including information about absences; and how they can assist such children in reentry, with an emphasis on the need to treat them normally. Classmates and peers should be given the opportunity to ask questions and should receive straightforward answers to their questions, including those about death.

The only experience that many children and adolescents have had with cancer is that of an adult in the family. They need to know that adult cancer is different from childhood cancer. They should be told that children, for the most part, respond very well to treatment. Also they should be reminded of the expectation that affected children will do well. [49] If affected children's prognoses are unfavorable, those around them should be told of the possibility of death if they ask, with emphasis on the fact that things are going well for now. [49]

Other children in the class may become worried also about their own physical symptoms. They may become afraid that the normal headaches and other body aches they experience mean they have cancer. They need to be reassured that cancer is a rare diagnosis and that every child has illnesses that are not related to cancer.

When presented with accurate, age-appropriate information, classmates can become returning patients' main source of support and sometimes can act to protect them from teasing. [15] Most professionals who work in pediatric oncology have seen male classmates and friends do such things as shave their heads as a show of support.

Several authors have noted that most incidents of teasing come from students in other classes. [15,34,39] It may be helpful to ensure that any teacher involved in the school reentry disseminate to other teachers any information that they receive. It may be feasible also for the professionals who perform the school reentry presentation to repeat it for other classes at a returning child's grade level or, at least, for all classes that an adolescent will attend.

### *School Reentry Plan*

Several other resources provide an appropriate template or framework from which to start in putting together a school reentry plan. These include

- Deasy-Spinetta P, Irvin E, eds. *Educating the child with cancer*. Bethesda, MD: American Cancer Society, 1993. Excellent all-around book for parents of children with cancer, as it also discusses education rights and long-term effects of cancer and its treatment. [50]
- Chambers A, Klinck A, Rynard D. *Helping schools cope with childhood cancer: current facts and creative solutions.* Ontario, Canada: Pediatric Division of Victoria Hospital, 1996. Provides a very good, comprehensive overview of issues of childhood cancer and was written with teachers in mind. [35]
- Nessim S, Katz, E. *Cancervive teacher's guide for kids with cancer*. California: Author, 1995. Well-done, small booklet written specifically for teachers. [32]
- Rolsky J. *Your child has cancer: a guide to coping.* Philadelphia: Committee to Benefit the Children, St. Christopher's Hospital for Children, 1992. Good all-around book; gives parents specific information related to all aspects of the experience of childhood cancer. [49]
- Schulz C. *Why, Charlie Brown, why?* California: Paramount Pictures, 1990. Great video that provides much useful information about childhood cancer in a very entertaining format for children in grades kindergarten through 3 or 4. [51]
- Sexson S, Madan-Swain A. School reentry for the child with chronic illness. *J Learn Disabil* 1993;26:115–125. [30]
- Rynard D, Chambers A, Klinck A, Gray J. School support programs for chronically ill children: evaluating the adjustment of children with cancer at school. *Child Health Care* 1998;27:31–46. [25]

Whatever combination of peer education and teacher education is used in a school reentry plan, one must keep in mind that every child is an *individual* with individual needs as regards school reentry. Although certain information always should be presented, the needs of returning children should be foremost in rendering school reentry a positive experience. Communication among the school, parent, and school liaison should be frequent in the days after school reentry to ensure that any problems or questions can be handled quickly. School liaisons should clarify also to school personnel that they can be contacted at any time in the future with questions or concerns regarding affected children.

### *Federal Laws Protecting the Educational Rights of Children with Disabilities*

Three federal laws protect the rights of children between the ages of 3 and 21 and having disabilities that impede their ability to benefit from their educational environment. These laws are the Individuals with Disability Education Act (IDEA); the Rehabilitation Act (section 504); and the Americans with Disabilities Act (ADA). These laws apply to every level of education, from infant and toddler to college and vocational education, and they guarantee every citizen the right to education regardless of physical, mental, or health impairment. Although these laws are federal, local and state governments interpret and implement them differently. [52] It is important that parents contact their state's department of education for guidelines governing the ways in which these laws are implemented.

Any services needed by children in school, such as special education or classroom accommodations, have to be formalized with a written plan using the IDEA or section 504 of the Rehabilitation Act. The written, signed plan will protect affected children's rights and provide documentation needed by parents if the services are not provided appropriately.

#### Individuals with Disabilities Education Act

The IDEA is a revision of an earlier law, PL 94-142 (Education of the Handicapped Act). The IDEA is a federal law that establishes a federal grant program to assist states in providing a free, appropriate public education, which includes special education and related services, to meet the unique needs of all disabled individuals between the ages of 3 and 21 [34 Code of Federal Regulations (CFR), Sec. 300.1(a)]. Additionally, such individuals' education must be provided in the least restrictive setting; “to the maximum extent appropriate, children with disabilities shall be educated with children who do not have disabilities” (34 CFR, Sec. 300.550-.556). Special education is defined as “specially designed instruction, at no cost to the parents, to meet the needs of a child with a disability” (34 CFR, Sec. 300.17). To receive special education under provisions of the IDEA, affected children must meet criteria for classification under one of several categories: mental retardation, hearing impairment, vision impairment, speech or language impairment, serious emotional disturbance, autism, deaf-blindness, traumatic brain injury, specific learning disability, orthopedic impairment, other health impairment, or multiple disabilities.

In the authors' experience, most children with diagnosed cancer are eligible for services under the category *other health impairment*, defined as “a child who has limited strength, vitality, or alertness due to chronic or acute health problems, such as heart condition, tuberculosis, rheumatic fever, nephritis, asthma, sickle anemia,

hemophilia, epilepsy, lead poisoning, leukemia, or diabetes which adversely affects educational performance" (34 CFR, Sec. 300.7).

Special education includes services ranging from simple classroom accommodations in a regular classroom to all-day placement in a resource room environment to instruction in the home, hospital, or other institution. Related services means transportation, corrective, and other supportive services that are required for children with a disability to benefit from special education. These include audiology and speech pathology; psychological services; physical and occupational therapy; recreation; counseling services; school health services; social work services in schools; and parent counseling and training (34 CFR, Sec. 300.16). Classroom accommodations that affected children may receive include use of a scribe or tape recorder to take notes; shortened class or homework assignments; provision of information instead of copying from a board or book; preferential seating; more time for tests or written work; oral testing; and permission to leave class early to avoid accidental injury caused by travel through crowded hallways.

To receive these services, qualifying children must be referred through a parental letter to the school's principal or to the special education director for the school district. Once the referral is made, the school system has a certain amount of time to evaluate such children or to review the evaluation performed by other agencies.

If children with cancer have received neuropsychological, physical, or occupational therapy evaluations while in the hospital, the school liaison can provide these reports to the appropriate school personnel, with parental permission. A letter from an involved physician outlining diagnosis, course of treatment, and any illness- or treatment-related impairments also is helpful. For those obtaining services under the category *other health impairment* or *orthopedic impairment*, the school system may have a form for physicians to sign verifying such impairments.

If children are deemed eligible for special education services, a meeting will be scheduled to design their individual education plan (IEP). The meeting should include, at the least, the parents, any teacher involved, a school administrator, and others involved in such children's care. It is advisable to have someone from the hospital, probably the school liaison, at the meeting to ensure that the pertinent aspects of the children's medical care, illness, and any transient or chronic impairments are well understood by the IEP team. The IEP constructed at the meeting should consist of certain elements [10]: present level of academic and cognitive functioning and statement of needs as identified by assessments; the annual goal and objectives, including procedures for evaluating whether the objectives are met; and educational placement and the amount of time allotted for participation in the regular classroom.

The plan also should include a statement regarding ability to participate in the state- or district-wide achievement tests and a statement regarding accommodations needed. If affected children will not be participating, a statement should indicate why the test is not appropriate and how these children will be assessed. A statement should address also the need to take Plan A of the Scholastic Achievement Test (allowing the student to take extra time with the test). Also necessary is a statement of transition services.

After the IEP is signed by all participants at the meeting, it becomes a legal document that, by law, the state is required to carry out as written. Parents should keep a file or binder of all appropriate documents, including the IEP, and a copy should go into returning children's medical records. The goals and objectives of the IEP are reviewed annually, and the IEP is rewritten if necessary; every 3 years, the child is reassessed. If, at any time, parents or any other member of the IEP team call for another IEP meeting, it can be scheduled to reassess a child's placement and services.

The IDEA mandates early intervention services for infants and toddlers who are either disabled or at risk of developmental delays. These services are provided either by school systems or by the state health department. The law requires that services be provided to affected children and their families. Rather than an IEP, an individual family service plan is written.

*Transition services* are defined in the IDEA (34 CFR, Sec. 300.29) as

> "a coordinated set of activities for a student, designed within an outcome-oriented process, that promotes movement from school to post-school activities, including post–secondary education, vocational training, integrated employment (including supported employment), continuing and adult education, adult services, independent living, or community participation. The coordinated set of activities must be based on the individual student's needs, taking into account the student's preferences and interests; and include needed activities in the areas of instruction, community experiences, the development of employment and to other post-school adult living objectives and, if appropriate, acquisition of daily living skills and functional vocational evaluation."

Specifically, 34 CFR, Sec. 300.347(b), requires that, beginning at age 14, each student's IEP include specific transition-related content and, beginning no later than age 16, a statement of needed transition services. Additionally, 34 CFR, Sec. 300.344(b)(3), requires that for the IEP meeting at which transition services are discussed, the school system must "invite a representative of any other agency that is likely to be responsible for providing or paying for transition services" (e.g., a representative from the department of vocational rehabilitation). The purpose of transition services is to provide linkages to help affected students, parents, the school system, and community agencies to work in an organized effort toward meaningful employment and a quality adult life for students with disabilities. [10] Although section 504 of the Rehabilitation Act of 1973 does not mandate transition plans, the 1998 amendments to the Rehabilitation Act do facilitate access to resources for transition, such as the Council for Independent Living and vocational rehabilitation services.

**Rehabilitation Act of 1973**

Section 504 of the Rehabilitation Act of 1973 (re-authorized in 1998) is not an education law or a federal grant program. It "clarifies that no individual with a disability in the United States, shall, solely by reason of his or her disability, be excluded from the participation in, be denied the benefits of, or be subjected to discrimination under any program or activity receiving Federal financial assistance or any program or activity conducted by any Executive agency" (34 CFR, Sec. 104.4). *Program* or *activity* is defined as including "all operations of a local education agency, system of vocational education, or other school system." [53] This law applies also to colleges, universities, and private schools that receive federal funds. Under the provisions of this law, the definition of *disability* is broader: "a physical or mental impairment which substantially limits one or more of such person's major life activities, such as learning; a record of such an impairment; or being regarded as having such an impairment."

The pertinent disability is not required to affect school performance adversely, and affected children do not have to come under the umbrella of special education to receive services. All persons with diagnosed cancer are eligible to receive services under section 504. [53] Each institution should have a section 504 coordinator who oversees compliance with this law. For affected children to receive services, a meeting similar to that for an IEP is conducted, and the needed services are written in the form of what is called a *504 plan*.

In addition to stipulating conditions in academic settings, the Rehabilitation Act prohibits discrimination in employment practices; program accessibility; health, welfare, and other social services; nonacademic and extracurricular activities, including clubs; counseling services; transportation; and health services. [10]

**Americans with Disabilities Act**

The ADA of 1990 provides a wider range of protection for all persons with disabilities. All persons with diagnosed cancer, even long-term survivors, are eligible for protection under the provisions of the ADA. It prohibits discrimination against persons with disabilities and applies to all state and local agencies (not just those receiving federal funds), including private businesses. The ADA not only prohibits discrimination against persons with disabilities but requires that persons with disabilities receive "reasonable accommodation." Although it is not an education law, its provisions do apply to education, including nonsectarian private schools. It provides a second layer of protection, in addition to section 504, to ensure that public schools provide reasonable accommodations for students with disabilities. [53]

***Stratification of Disability Levels***

At least one author has identified a need for staging the effects of disease or treatment on cognitive functioning, in much the same way in which disease staging is used to determine appropriate treatment.[54] In this chapter, we propose that a level of disability be identified at the time of school reentry and throughout affected children's scholastic career to identify clearly the resources needed and to discern which children are at risk of long-term learning problems. School reentry needs are determined by the level of disability.

**Level 1: No Premorbid Disability and No Chronic Illness– or Treatment-Related Impairment**

Children at level 1 may have only transient problems related to school absence, fatigue, and restrictions in physical activity. A more visible change will be loss of hair. They may not be able to play on the playground or participate in sports or physical education for a period. They also may need to make up work missed in their absence and will have future absences because of follow-up clinic visits at regular intervals. Because such children are unlikely to have received previous special services, school reentry planning should include discussion with affected parents about their rights and their children's rights under the provisions of the IDEA and section 504 of the Rehabilitation Act, so as to obtain any needed services or classroom accommodations. The school liaison should give them information about the referral process and assist them with meeting with school personnel. The liaison also should provide school personnel with information about affected children's treatment or follow-up schedules and frequency of future absences. School reentry may not mean coming to school for 5 days per week but may mean a modified schedule of only half-days or a 2- to 3-day week. Use of home-bound services can continue to supplement educational services for such children when they cannot attend school. Shortened or modified assignments also may be used to assist these children with staying abreast of schoolwork. Affected children's IEP or 504 plan should include any services or accommodations that they need to complete the school year successfully.

**Level 2: Premorbid Disability and No Chronic Illness– or Treatment-Related Impairment**

Premorbid disabilities may include attention deficit–hyperactivity disorder; learning disability; hearing, vision, or speech impairment; mental retardation; behavioral problems; or affective disorders. Any premorbid learning or adjustment problems, along with the school absences related to affected children's treatment, will render school reentry more difficult.[12,15,20,22,30,37,55] Even such transient problems as fatigue may be overwhelming to already functionally impaired children or adolescents.

Children and adolescents in this category may have had some level of special education services or special classroom accommodations and may have an IEP or a section 504 plan. Possibly, previous services were, or may have become, inadequate or inappropriate. School reentry planning for such children should include contact with professionals at the hospital, such as a psychologist or the school liaison, who can review children's previous services and make recommendations for improvement, if necessary. Neuropsychological testing also may be helpful or necessary to assist with school reentry.

Such children may need a higher level of support, such as aides or tutors, to assist them during class time. Also, plans are necessary for use of home-bound services when these students cannot attend school.

**Level 3: No Premorbid Disability but Chronic Illness– or Treatment-Related Impairment**

Chronic impairment usually is associated with central nervous system–directed therapy for ALL or brain tumors.[8,38,56] Because children at level 3, like those at level 1, are unlikely to have received special services, school reentry planning should set aside time to discuss with parents their rights and their children's rights within the scope of the IDEA and section 504.

Chronic impairment, as the name implies, is likely to be more permanent or persistent. School reentry planning, as one would expect, is more complex for chronically impaired children and may involve coordination with other professionals in a multidisciplinary format. Such children's appearance also will differ from that of their peers. Visible changes go beyond hair loss and may include the use of a wheelchair or use of assistive technology, such as a magnifier for reading, a hearing aid, or an auditory trainer.

For such impairments as hemiparesis or vision, hearing, or speech impairment, a qualified professional should perform an assessment to determine the level of therapy or support needed by affected children. Neuropsychological assessment also should determine the level of cognitive functioning and should document any learning disabilities or deficits (e.g., slow processing speed, visual and perceptual deficits, and problems with memory or attention). These evaluations probably will be performed at the hospital treating the child, but outside rehabilitation professionals may be involved as well.

School reentry planning must address the special educational services or classroom accommodations certainly needed by chronically impaired children. It may be best to start with a conference of all professionals who have evaluated such children to plan for the specific services they should receive in school. Parents should be included at this conference and, at that time, a release of information can be obtained from them so that all evaluation reports can be sent to the school system after such children have been referred for special services. This meeting should produce a list of specific recommendations that can go into affected children's IEP or section 504 plan.

Affected children may need occupational therapy, physical therapy, or speech therapy or a special teacher for the visually impaired or hearing impaired to assist in a regular classroom. Full-time placement in the regular classroom may not be possible, so such children may have to spend part or all of their days in a resource room environment where the student-teacher ratio is lower and each child can work at his or her own level.

Affected children also may not be able to attend school every day or for an entire day. As with more transient impairments, an alternative educational plan using home-bound services must be developed to ensure access to educational services during frequent or prolonged absences. Once recommendations are made, a referral for services should be made as per aforementioned guidelines. It would be advisable to have someone, such as the school liaison, at the meeting to discuss any pertinent medical issues, answer any questions from school personnel, and act as a child-parent advocate.

All recommendations regarding therapy, alternative class placement, and alternative educational services should be outlined clearly in the IEP or section 504 plan. Once they have been written and approved by the parents, follow-up should continue to ensure that affected children receive services as outlined in the plan and to make any necessary changes required by changes in the children's level of ability.

One additional issue that must be raised with these children is the fact that these changes in cognitive or physical abilities may necessitate changes in expectations for the future. Plans for college may change to plans for vocational training or for a 2-year degree, and plans for an athletic scholarship or career may have to be set aside. Concomitantly, both parents and children may need support to develop acceptance of their need to reset expectations. All parents want their children to live up to their potential, but when that potential is changed by a cognitive or physical impairment, parents may have difficulty in understanding and accepting that what was once possible for their children is no longer within reach. They experience disappointment, even grief, for the loss of their premorbid children. Just as in other grief experiences, denial can be a factor, and some parents may expect their children simply to work harder. They sometimes do not understand or accept that this attitude will only continue to frustrate children who probably already are working harder and who themselves do not understand why they are experiencing more difficulty with some subjects.

Psychologists and physicians who evaluate and treat chronically impaired children must be aware of these possible problems and should carefully explain to both parents and children any illness- or treatment-related impairment, how the impairment changes affected children's ability to function cognitively or physically, and how these changes will affect plans for these children's future. The psychologist or physician should assess parents' and children's understanding and acceptance of this information and provide further information and counseling as necessary to assist with this process. In follow-up, they should maintain discussion with both parents and children and assist them as necessary in finding a new direction for their children's future. Identifying the latent objectives underlying the manifest goals can serve as the means by which a family and child can identify new goals to achieve the same objective. For example, the manifest goal of an athletic scholarship that served the latent objective of financing college may be replaced by the goal of obtaining alternative scholarships through cancer survivor agencies, thereby satisfying the objective of paying for college.

**Level 4: Premorbid Disability and Chronic Illness– or Treatment-Related Impairment**

Children at level 4 may be the most impaired, as problems related to the premorbid condition may be exacerbated by the illness or there may be treatment-related impairment. As with children in the other levels, it is imperative that plans be made for alternative educational services for such children during absences that occur while they are in treatment. Again, home-bound services can be used when affected children are unable to attend school.

School reentry planning should include a review with the parents of children's previous special education services or classroom accommodations and discussion of any additional or more intensive services needed because of chronic illness– or treatment-related impairment.

As is scheduled for those at level 3, a conference of professionals involved in affected children's care should include the parents to discuss specific recommendations for meeting their children's needs. If a previous IEP or 504 plan is in effect, it can be reviewed at this meeting or the parents can call school personnel to arrange a

meeting for revision of the plan. If no previous plan exists, a letter of referral for services (as mentioned) would be appropriate.

**Phase 3: Follow-Up Contact**

***Initial Follow-Up***

In the weeks and months after reentry to school, school personnel and the school liaison should continue to communicate frequently to assess how affected children are adjusting to the school environment. Any further assistance should be given as necessary. If such children have an IEP or section 504 plan, the school liaison can assist the parents in ensuring that their children are receiving the services according to the plan.

***Long-Term Follow-Up***

In our model of school reentry, follow-up for children with cancer continues for years, through high school and college or vocational training. Long-term follow-up is necessary because of the long-term or late neuropsychological effects of therapy for cancer. As defined by Mulhern, [38] there are “pathological changes in the child's central nervous system (CNS) secondary to cancer or its treatment that are manifested by stable changes in the child's behavior.” For our purposes, *behavior* includes cognitive functioning and academic achievement.

Not all children treated for cancer receive treatment that affects the central nervous system or its functioning. [57] Significant physical and psychosocial effects relate to diagnosis and treatment of any childhood cancer, but the transient impairments resolve with few or no long-term sequelae. [57] However, 50% of children treated for cancer, specifically those with ALL or brain tumors, receive cranial radiotherapy (CRT) or systemic or intrathecal methotrexate. Several recent studies have shown that such children are at increased risk of neuropsychological late effects from their illness or from treatment that significantly limits their attainment of educational and vocational goals.[58] The greatest risk for these children appears to be related to age at the time of diagnosis and treatment and gender; female patients receiving CRT are at greater risk than are male patients.[38] Other factors associated with greater risk are total dose of radiation, age at the time of CRT, administration of CRT and intrathecal methotrexate in combination, and additional intensive therapy for relapsed disease. [58]

Testing of long-term survivors of ALL and brain tumors indicates that cognitive deficits usually do not begin to appear until 2 to 4 years after the start of treatment and that the magnitude of the deficit increases with time after treatment.[38,57,58 and 59] Psychometric scores also decline progressively over 2 to 4 years.[8,38,57,60] Cognitive deficits seen on neuropsychological testing include deficits in sequential memory, arithmetic, processing speed, visuomotor integration, attention and concentration, and fine motor coordination.[8,38,59,61,62] The decline in standard scores usually is not representative of a progressive deterioration of abilities but of a significant slowing of the rate of development of abilities relative to the rate demonstrated by others in the patient's age cohort. [8] This information, combined with the facts that younger children have greater deficits and that specific abilities associated with frontal cortex function are impaired led Armstrong and Horn [8] to propose a model of developmental emergence of deficits. According to this model, treatment with central nervous system–directed therapies interferes with the normal development of the frontal cortex, thus interrupting or delaying functions that would have emerged as part of the normal development course.[8,60] Structures that are developed prior to treatment remain intact. This model helps to explain why a child's performance on tests of neuropsychological function is within normal limits during a given year but becomes significantly impaired a few years later.

The fact that these neuropsychological deficits are not readily observable [60] suggests that even in the light of average academic performance, neuropsychological abilities should be assessed at regular intervals, according to a plan of surveillance. [38] Often, school, and medical professionals do not have information about the premorbid functioning of children and do not consider the fact that some of such children were above average to superior in function before the administration of central nervous system–directed therapies, and average performance in these children may represent a slowing of the acquisition of skills. [54] Although they appear to be doing well, such students actually may need special accommodations or services to retain as much of their previous learning potential and scholastic performance as possible.

Provision of special educational services or classroom accommodations for children with cancer may be complicated, because their disabilities do not always conform to the discrepancy model used by most U.S. school systems to determine eligibility for services under the category *specific learning disability*.[61] This model uses a formula that mandates a discrepancy of one standard deviation (15 points) between measured intelligence quotient and measured academic achievement. For children with cancer, this discrepancy may not show up for years, during which time meaningful intervention may have been lost. [60] For that reason, many children with cancer receive services under the IDEA category *other health impairment* or under section 504 of the Rehabilitation Act. These categories for eligibility do not require the use of a discrepancy model.

School personnel, including school psychologists, often have little or no experience with children with cancer and do not understand the potential impact of treatment for ALL or brain tumors. Problems with attention or memory may be interpreted by school personnel as laziness, lack of motivation, or other emotional difficulties. [63] School psychologists also may lack experience with the kinds of neuropsychological assessment needed to define the cognitive deficits experienced by such children. Therefore, it is important either that the child be assessed by a pediatric psychologist or neuropsychologist through the auspices of the hospital or that the psychologist consult with the school psychologist to assist with the assessment process.[38,58,60] Mulhernet al.[58] proposed guidelines for the assessment of school problems to assist professionals with this process. Armstrong and Horn[60] also suggested an assessment approach. They recommended that affected children be tested at 12- to 18-month intervals. They also urged that, in addition to neuropsychological tests, curriculum-based measures be used to track the progress of individual children.

The foregoing findings only corroborate the need for pediatric oncology professionals, parents, and school personnel to work together to evaluate the school performance and neuropsychological abilities of children at risk and offer appropriate assistance. One author even suggested that parents be asked to bring their children's report cards to the clinic on a regular basis so that school performance could be assessed. [64] To facilitate this vigilance, the long-term neuropsychological effects of disease and treatment must be explained carefully and explicitly to involved parents and to older children who can participate in decision making. [38] For school personnel to become full partners in this process, they also should have access to such information.

As stated, professionals also need to assess affected parents' and children's understanding and acceptance of any changes in functioning and the need to reset expectations for children's future. Further information and counseling should be provided as necessary to help parents and children with this process. The basic principle is to assist family and child in finding alternative paths to broader life objectives, such as personal productivity, meaningful interpersonal relationships, financial stability, recreational enjoyment, and personal identity.

***College and Vocational Training***

Not all high-school graduates can or will attend college, but the presence of any learning disability related to disease or treatment should not keep young adults from considering the possibility of college attendance. Colleges and universities must accommodate students with disabilities, according to provisions of the Rehabilitation Act of 1973 and the ADA. Having an IEP or section 504 plan in high school will provide the documentation necessary to assist students in obtaining appropriate services in college. If students are attending college when disabilities are discovered or when they become severe enough to affect school performance, appropriate assessment will provide the documentation.

Many colleges have a person designated to work specifically with students with disabilities. To help students to determine which college may have the best program for a specific student, several books list colleges and universities that provide specialized programs (e.g., Peterson's *Colleges with Programs for Students with Learning Disabilities or Attention Deficit Disorders*).[65] Those students who do not want to attend a 4-year college or who cannot because of their level of functioning have alternatives, including 2-year training programs through community colleges and vocational or technical training. Sheltered workshop programs are available for those who are more severely impaired. As mentioned, a plan for transitional services should be included in an adolescent's IEP or section 504 plan.

***Terminally Ill Children***

School reentry and intervention programs emphasize the hopeful aspect of childhood cancer. However, sometimes children's cancer progresses, all curative treatment has been exhausted, and affected children enter the terminal phase. When should school services end? Davis [41] chose to answer the question by looking at

the range of school services available.

Terminally ill children probably have been in the chronic phase of treatment and have been attending school. [40] School participation can change from school attendance to home-bound services or any combination of the two to accommodate terminally ill children's physical problems or minimize their discomfort. Continued school participation is very important to the emotional well-being of children, even terminally ill children, and it may be one of the few normal activities in which such children can continue to participate. [31,66] This wasmade clear also by Lansky et al.[64] in a review of absenteeism in children with cancer. These authors found that children attended school one-half the time during the year they died. It is vitally important that teachers continue to see such children for as long as the children desire. If contact with affected children is stopped suddenly against their wishes, they will feel “abandoned, lonely, and helpless.” [40] At some time, academics no longer will be appropriate, and teachers may want to engage such children in other activities. [40]

Another issue is support for teachers and classmates. Affected teachers will be trying to support a terminally ill child's classmates but will need support also. School or hospital mental health professionals may have to be consulted for assistance. Communication from the school liaison should be frequent, answering questions the classmates may have about such children and their condition. The American Cancer Society's *Back to School: A Handbook for Teachers of Children with Cancer* outlines plans for dealing with the terminal illness of a student. [31]

## CONCLUSION

It is important that the process of school reentry and intervention begin at the time of diagnosis and continue through the transition to college or vocational training and into adulthood. Educational intervention is one of the most exciting areas of research in pediatric oncology. It is also one of the most promising because, as survival rates increase, the quality of life of survivors becomes ever more important. Because education is necessary for successful entry into employment and adulthood, it is imperative that affected children and adolescents receive the services they need to realize their cognitive potential fully. Toward this end, it is imperative also that parents and professionals who work with children with cancer, including physicians, psychologists, and teachers, have a clear and thorough understanding of the educational issues involved. They must also be prepared and committed to work together as a team to provide the long-term, continuing intervention and follow-up needed.

Parents of children with cancer need information and support to become assertive and effective advocates for their children. As children grow to adolescence and young adulthood, they also must have this information and become advocates for themselves. Through an integrated, developmental approach to school reentry and continued school intervention, the survivor of childhood cancer can look more optimistically forward to successful adult life.

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# CARE OF THE DYING CHILD

JOANNE WOLFE
HOLCOMBE E. GRIER



## INTEGRATING PALLIATIVE CARE

Approximately 25% of children diagnosed with cancer eventually die of their disease. This makes cancer the leading cause of nonaccidental death in childhood. [1] High-quality palliative care is now an expected standard. [2,3,4] and [5] The American Academy of Pediatrics set the following as a minimum standard for pediatric palliative care: “Excellence in pediatric palliative care is essential for hospitals and other facilities caring for children. Program development in pediatric palliative care, along with community outreach and public education, must be a priority of tertiary care centers serving children.” [2]

The World Health Organization has defined palliative care as the active total care of patients whose disease is not responsive to curative treatment . . . [when] control of pain, of other symptoms, and of psychological, social and spiritual problems is paramount. [6] It may not be possible to determine whether a disease will be responsive to curative treatment, however, nor is it possible to determine which type of trajectory the dying process will take ( Fig. 51-1).[5] Some children may die suddenly and unexpectedly—for example, the child undergoing bone marrow transplant who experiences a treatment-related complication ( Fig. 51-1A). Others may experience a steady and fairly predictable decline ( Fig. 51-1B), such as the child with a progressive brainstem glioma after radiation therapy. Finally, other children with progressive cancer may experience fairly long periods of chronic illness punctuated by crises, one of which may prove fatal—although an entirely different problem may intervene to cause death (Fig. 51-1C). An example of this type of trajectory may involve a child with relapsed metastatic neuroblastoma who may be palliated long term, before experiencing a life-ending event.

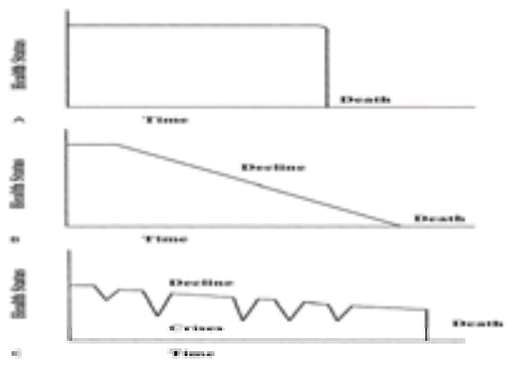



**FIGURE 51-1.** Prototypical death trajectories. **A:** Sudden death from an unexpected cause. **B:** Steady decline from a progressive disease with a “terminal” phase. **C:** Advanced illness marked by slow decline with periodic crises and “sudden” death. [Adapted from Field MJ, Cassel CK, Institute of Medicine (U.S.). Committee on Care at the End of Life. Approaching death: improving care at the end of life. Washington, D.C.: National Academy Press, 1997:29.]

When the care team focuses solely on life extending therapy, as opposed to maximizing quality of life, their goal can drive futile interventions that prohibit the patient from receiving optimal comfort care.[7] This is especially true when the institution of palliative care is viewed as an abrupt transition from life-prolonging to symptom-oriented care (Fig. 51-2A).[7] If palliative care is conceptualized as always being a part of the care paradigm ( Fig. 51-2B), the transition to predominantly comfort care can occur gradually and intuitively. This approach is supported by a survey of parents of children who died of cancer, which demonstrated that parents maintain dual goals of care concurrently.[8] Specifically, the majority of parents reported that during their child's end-of-life care period, their primary goal was to lessen suffering; at the same time, they reported that their primary goal of cancer-directed therapy was to extend life. Thus, optimal care of the dying child requires recognition on the part of the care team that it may not be clear when a child is dying, but if death is a likely outcome, palliative care should be a priority.

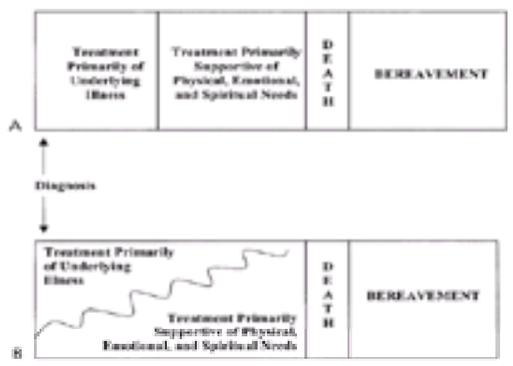



**FIGURE 51-2. A:** Abrupt transition to palliative care. **B:** Progressive transition to palliative care. (Adapted from Sahler OJ, Frager G, Levetown M, et al. Medical education about end-of-life care in the pediatric setting: principles, challenges, and opportunities. Pediatrics 2000;105:575–584.)

## DISCUSSING PALLIATIVE CARE

Optimal palliation requires the establishment of open and ongoing communication between all care team members, the child, and the family. Wolfe and colleagues [8] have shown that parents first recognize that the child has no realistic chance for cure more than 3 months after the primary oncologist realizes this fact. It is likely that there are yet unknown physician, parent, and child factors that contribute to this delay. Yet the study also showed that earlier recognition by the physician and parent that the child had no realistic chance for cure was associated with better integration of palliative care. Thus, early and ongoing discussions aimed at informing parents of the possibility of a child's death might be critical to easing suffering during the end of a child's life.

### Breaking Bad News

Much has been written about communication strategies for breaking bad news. [9,10] and [11] Recommendations have focused on ensuring privacy and adequate time; assessing the families' understanding of the condition; providing information simply and honestly; encouraging patients and parents to express their feelings and empathizing with them; and providing a strategy for approaching the situation and a summary of what was discussed. It is critical to end such discussions by assuring the family that there will be follow-up opportunities for ongoing discussions that address continued concerns.

### Introducing Palliative Care

It is less clear how to discuss palliative care with families. Recognizing the need to respect patients' wishes to maintain hope for cure or further life extension, Billings[12] strongly suggests that the terminal prognosis not be a part of the dialogue. Rather, he proposes the following statement when introducing the concept of palliative care to families: “Palliative care is a special service, a team approach to providing comfort and support for persons living with life-threatening illness and for their families. We are a nurse, a social worker, a chaplain, and physicians who work . . . to assure that you and your family receive excellent pain control and other comfort measures, get the information you want to participate in decisions about your care, receive emotional and spiritual support and practical assistance, obtain expert help in planning for care outside the hospital, continue getting good services in the community, and overall enjoy life as best as you can, given your condition. We try to coordinate and tailor a package of services that best suits your values, beliefs, wishes, and needs in whatever setting you are receiving care.” Lo and colleagues[13] have proposed several strategies to try to address some of these issues with patients and families. For example, they emphasize starting with open-ended questions such as What concerns you most about your child's illness? How is treatment going for you/your child and your family? As you think about your/your child's illness, what is the best and worst that might happen? What are your/your child's hopes (expectations, fears) for the future? These open-ended questions provide a means to explore the possibility of a child's dying.

### Discussing Palliative Care with Children

Very little is known regarding communication about palliative care with children with advanced cancer. An important consideration is the extent to which children should be included in the decision-making process. Knowledge of the developmental understanding of death should help guide this generally unexplored area ( Table 51-1).[14]

| Age range (yr) | Concept |
|---|---|
| Birth–2 | Death is perceived as separation or abandonment<br>Protest and despair from disruption in caretaking<br>No cognitive understanding of death |
| 2–6 | Death is reversible or temporary<br>Death is personified and often seen as punishment<br>Magical thinking that wishes can come true |
| 6–11 | Gradual awareness of the irreversibility of death<br>Specific death of self or loved one difficult to understand<br>Concrete reasoning with ability to see cause and effect relationships |
| Older than 11 | Death is irreversible, universal, and inevitable<br>All people and self must die, although latter is far off<br>Abstract and philosophical reasoning |

From American Academy of Pediatrics. Committee on Psychosocial Aspects of Child and Family Health. The pediatrician and childhood bereavement. Pediatrics 2000;105(2):445–447, with permission.

**TABLE 51-1. OVERVIEW OF CHILDREN'S CONCEPTS OF DEATH**

#### *Developmental Understanding of Death*

Most children learn to recognize when something is “dead” before they reach the age of 3 years, but at this early age, death, separation, and sleep are almost synonymous in the child's mind. As children grow to preschool age, they recognize that a dead person cannot function, but they are likely to believe that death is temporary. They still don't understand why people die, however; their egocentric reasoning makes them vulnerable to believing they can cause death with their thoughts or actions.

School-age children are problem solvers, with the beginnings of logical thought. During these years they normally acquire a much more complete understanding of death. By the age of 7 years, most children understand that death is irreversible, that everyone will die, that the dead do not function, and that people die from both internal and external causes. They are interested in the specific details of death, and in the latter part of this phase they are able to envision their own deaths.

As children become teenagers, their thinking about death is usually consistent with reality. They are ready to add to their complete definition of death the effect it has on other people and on society as a whole. Their future orientation makes it difficult for them to recognize their own deaths as a present possibility, although they can conceive this occurring at some point in the future.

#### *“Children Want to Know Their Prognosis”*

In general, children with a terminal illness appear to have a precocious understanding of the concepts of death and their personal mortality. [15,16,17] and [18] Studies have indicated that children with cancer want to know about their prognosis. In a survey of 50 children with cancer aged 8 to 17, 95% of patients wanted to be told if they were dying.[19] Although most of the children felt that treatment decisions were up to the physicians, 63% of the adolescents and 28% of the younger children wanted to make their own decisions about palliative therapy. Nitschke and colleagues [20] reported on their experience of including children aged between 6 and 20 years in a

“final stage conference” in which progression of disease, minimal chance of cure, imminence of death, and therapeutic options were discussed. These children appeared capable of making rational decisions about further therapy. [20] Others have suggested that children younger than 11 years may not be able to grasp these concepts.[21,22] The approach should be tailored to the individual child and family.

It is important to emphasize, however, that it is impossible to lie to a child and preserve a relationship that is built on trust and caring. According to Hilden and colleagues,[23] children will often know when they are dying and may feel tremendous isolation if they are not given permission to talk openly about their illness and impending death. Furthermore, it is now generally accepted that children give their assent in medical decision making. [24]

### *Communicating with Children*

In communicating with children it is important to stay open and receptive when the child initiates a conversation. “Teachable moments” may be fleeting, and an immediate response is necessary to capitalize on them. Recognize that many children communicate best through nonverbal means such as artwork or music. They may also be more willing to “talk things over” with puppets or stuffed animals rather than real people. Importantly, euphemistic expressions about death can be very confusing or even frightening for children (for instance, equating death with sleep may result in the child being afraid of going to bed).

### Resuscitation Status

Discussion of appropriateness of initiating cardiopulmonary resuscitation for children with advanced cancer is a very emotional topic. [25] For this reason, medical caregivers often avoid these conversations until respiratory or cardiac collapse seems imminent. [26] Clearly, parents would be better able to consider this decision if they were not in the midst of a crisis. Thus, advanced discussions regarding resuscitation status are strongly recommended.

We have often approached this sensitive topic by framing it as “in the worst case scenario we would like you to consider whether your child should undergo cardiopulmonary resuscitation if we believe she or he has an irreversible problem.” This approach along with reassurance that a life-threatening event is not imminent enables parents to maintain hope while facing this decision. It is also helpful to reassure parents that should the child's condition improve, this status would be reconsidered. Even if a family is unable to make a decision about resuscitation status during the initial conversations, it is helpful for them to have heard that they may have to face this decision in the future.

Careful thought should be placed on the exact words used during a discussion regarding resuscitation status. Parents often think that agreeing not to resuscitate is choosing death over life for their children. It is helpful to explain that it is the uncontrolled cancer that would cause the death. To some parents, the phrase “do not resuscitate” implies that if these interventions were performed, they would be successful. Experience tells us that among children with advanced cancer, the likelihood of a patient being extubated and leaving the hospital is very low. Thus, when approaching families about this issue, it is preferred to use the phrase “do not attempt resuscitation (DNAR).”[27] Importantly, it is not always possible to know for certain whether a change in clinical status that necessitates resuscitation efforts is an irreversible event. For example, apnea related to administration of seizure medications may well be a reversible event. Thus, documentation should always specify under which circumstances a DNAR order is applicable. For outpatients, it is helpful to have an accompanying letter that briefly summarizes the patient's medical condition and documents the details of the resuscitation status discussion. In several states there is also an official DNAR verification form, such as the Comfort Care Form in Massachusetts, which permits emergency medical teams to honor DNAR orders written in the hospital or clinic.

### Location of Care

Studies have found that a little more than one-half of children with progressive cancer die at home. [28,29] Future efforts need to be aimed at better understanding why some families choose to remain primarily at home, whereas other parents prefer their child to die in the hospital. Some have suggested that family adjustment after the death is better if the child dies in the home.[30,31] The beneficial effects of care in the home on parental functioning have been attributed to fewer feelings of helplessness and the broad opportunity for family intimacy offered by home care. Given the retrospective nature of these studies, however, cause and effect relationships cannot be established with certainty. Parents who choose home care may have premorbid differences with respect to personality traits and coping. Others have found family relationships to be better when the child died in the hospital. [32] Although many have suggested that most children prefer to die at home, this, too, has not been systematically evaluated.

Regardless, it is critically important to discuss preferences regarding the primary location of care as early as possible. Options include inpatient care or home care with or without the support of a home care team. Presently, more and more hospitals are developing palliative care services, the impact of which has yet to be evaluated.[33,34,35] and [36] Home care teams might include a visiting nurse association, bridge programs, or hospice. There are very few inpatient pediatric hospice units. A parental decision to care for their terminally ill child at home involves the consideration of medical, psychological, social, and cultural factors together with such practical considerations as the availability of respite care, physician access, and financial resources. [35] Furthermore, whatever the decision is regarding the primary location of care, families should be reassured that they can change from one option to another and that the primary team will remain closely involved. [37]

There are special challenges that we face regarding home care for terminally ill children. Specifically, because death from chronic illness in childhood is uncommon, the low census makes it difficult to maintain a staff of caregivers experienced in pediatric issues. Pediatric programs often face financial difficulties and are hard to sustain in the long term. Furthermore, caregivers skilled in the care of dying adults lack the expertise to deal with the unique medical and psychological needs of children.[38] In addition, communication between the primary hospital- or clinic-based team and the home care team is often suboptimal because these medical caregivers are not familiar to one another. Finally, parents may be reluctant to accept hospice because they equate this with giving up.

When families are able to embrace hospice, however, assistance from these programs can be invaluable. [37] Support bythe hospice team may include not only the nurse, physician, and home health aide but also the social worker, chaplain, and volunteers. [39] Optimal children's hospice care incorporates physical care for the child, home care, pain and symptom management, psychosocial support for child and family, respite care, staff support for health care providers, and special services such as transportation. Importantly, hospice programs are required to provide bereavement follow-up for family members. It is helpful to understand, however, that to be eligible for the hospice benefit, the physician must consider the child's life span to be no longer than six months. [39] Furthermore, reimbursement mechanisms for hospice differ from typical home care. Specifically, these programs are reimbursed less than $100 per day per patient. Because philosophically hospice programs encourage a natural, simple, and cost-effective approach to end-of-life care, this reimbursement mechanism is not usually problematic. For children, however, there needs to be greater flexibility, because with current practice they often continue to receive fairly intensive and often expensive care until the very end. Families should not have to choose between these two approaches based on cost.[2]

## CANCER-DIRECTED THERAPY

Many families opt for continued treatment of the underlying cancer even when there is no realistic prospect for cure. [8,40] Their rationale might include hoping for a miracle, a desire to extend the duration of the child's life even though there is no possibility of cure, or to palliate symptoms related to progressive disease. In discussions of treatment options with families, we often state the following: “The very nature of miracles is that they are rare. However, we have seen miracles, and they have occurred both on and off treatment.” In other words, a child does not have to continue on cancer-directed therapy to preserve hope, especially when the therapy significantly impacts the child's remaining quality of life. Generally, decisions regarding continued cancer-directed therapy need to be carefully considered, weighing the potential for life extension and the impact on quality of life.

### Palliative Chemotherapy

An inherent conflict exists in terminal cancer care between life-extending measures and efforts to minimize global suffering. Palliative cancer therapy can prolong life and lessen suffering. Alternatively, administration of treatments may result in increased numbers of physician-patient interactions, visits to clinic, admissions to the hospital, and most important, treatment-related complications requiring augmentation of supportive care. Nevertheless, there are a number of studies that show improved quality of life among adult patients receiving chemotherapy compared to those who were not. [41,42,43,44] and [45] Several possible reasons for this include placebo effect, provision of hope, or increased medical attention associated with being on treatment. The role of palliative chemotherapy in children has not been studied. The benefits may depend on the developmental stage of the child and his or her awareness of disease state. For example, increased interactions with medical personnel may outweigh any improvements in quality of life for the child. Parents may also have differing views on the role of continued cancer-directed therapy. Wolfe and colleagues[8] found that only 13% of parents reported that the primary goal of cancer-directed therapy for their child during the end-of-life care period was to lessen

suffering. The majority of parents maintained a primary goal of extending life. Communication about this issue must be very clear and tailored to the individual family.

Several agents have been shown to be well tolerated in children and to have some antitumor effect. For example, oral etoposide has antitumor effect with limited toxicity in children with refractory neuroblastoma, germ cell tumors, brain tumors, rhabdomyosarcoma, and other solid tumors. [46,47,48,49,50,51,52] and [53] Relapsed acute lymphoblastic leukemia may be temporarily controlled with regimens including vincristine, methotrexate, prednisone, and 6-mercaptopurine. The decision about whether to continue cancer-directed therapy must carefully balance considerations of efficacy, potential treatment-related complications, and psychological impact.

### Phase I Trials

The goal of phase I research is to determine the toxicities and maximum-tolerated doses of an investigational drug or drugs. Yet Daugherty and colleagues [54] found that only one-third of adults enrolled in a phase I trial were able to state the purpose of the trial. They also found that cancer patients who participate in phase I trials are strongly motivated by the hope of therapeutic benefit. Altruistic feelings appear to have a limited and inconsequential role in motivating patients to participate in these trials. Yet overall, the chance of tumor response in phase I trials is low, ranging from 4% to 6%. [54,55] In children the response rate is similar. [56] It is important to note, however, that the chance of fatal toxicity is also low, at approximately 0.5%. [55,56] In general, physicians tend to assume more positive potential benefit from experimental chemotherapy than statistics would warrant.[54] Although these biases are not presented to the family with any intention of doing harm, they may make the informed consent process exceedingly difficult and potentially raise serious ethical questions. [57]

Similar to discussions about palliative chemotherapy, it is critical to ensure effective communication when discussing phase I therapy for children with advanced cancer. Furthermore, it is strongly recommended that children give their assent to participation in clinical trials. [58]

### Radiation Therapy

Although estimates vary, approximately one-half of all courses of radiation therapy are delivered with palliative intent. [59] Palliative radiation is given with the intent of relieving symptoms, and complete elimination of the tumor is unnecessary. Larger fraction sizes over shorter time frames can be used in most cases, as late-arising complications are not of major concern.[60] Munro and Sebag-Montefiore[61] have devised the concept of *opportunity cost*—that is, what the time spent on the treatment of a dying patient costs in terms of lost opportunities in his or her remaining lifespan. Common indications for palliative radiation include the following:

- Pain relief from bony or pulmonary metastases and tumors causing nerve root and soft tissue infiltration
- Control of bleeding
- Control of fungation and ulceration
- Relief of impeding or actual obstruction—for example, of the large airways
- Shrinkage of tumor masses causing symptoms—such as brain metastases, skin lesions, and other sites
- Oncological emergencies—such as spinal cord compression, superior vena caval obstruction. [59]

In the absence of symptoms to palliate, there is probably little value in giving treatment unless it is apparent that significant problems are incipient.

## SYMPTOM MANAGEMENT

Wolfe and colleagues[29] recently demonstrated that according to their parents, children with terminal cancer experience a high prevalence of symptoms during the last month of life, with fatigue, pain, and dyspnea resulting in significant suffering ( Fig. 51-3). Parents also reported limited success in treating symptoms. Aggressive symptom management is a priority in patients with advanced cancer. Symptoms that are out of control should be considered a medical emergency requiring direct evaluation of the patient and immediate implementation of interventions.

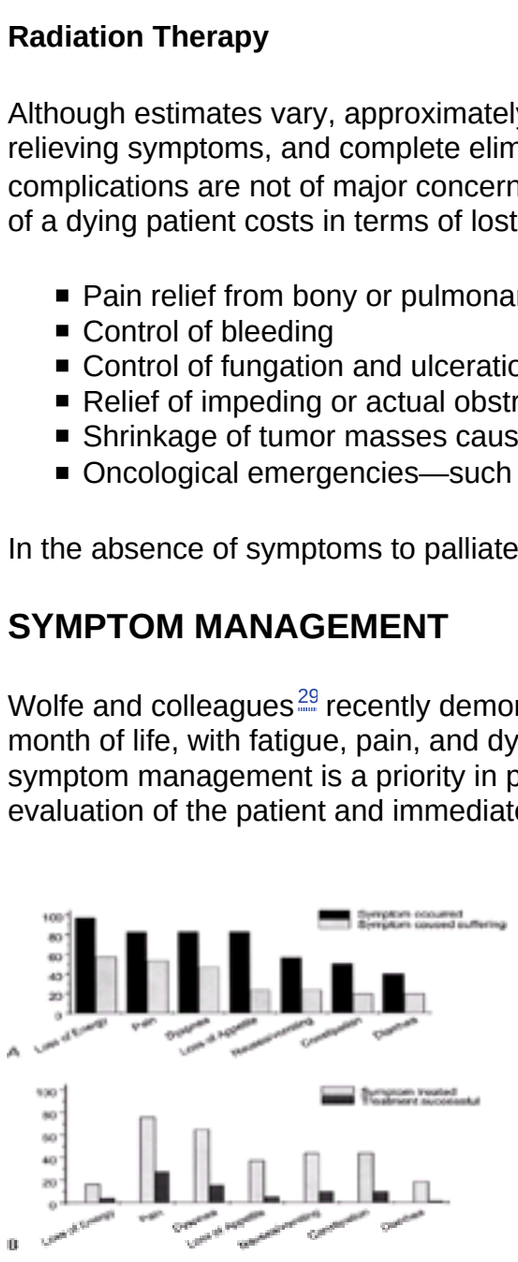



**FIGURE 51-3.** The degree of suffering from and the success of treatment of specific symptoms in the last month of life. **A:** The percentages of children who, according to parental report, had a specific symptom in the last month of life and who had “a great deal” or “a lot” of suffering as a result. **B:** The percentages of children who, according to parental report, were treated for a specific symptom in the last month of life, and in whom treatment was successful (rather than “somewhat successful” or “not successful”). (Adapted from Wolfe J, Grier HE, Klar N, et al. Symptoms and suffering at the end of life in children with cancer. N Engl J Med 2000;342:326–333.)

### Fatigue

Fatigue is the most common symptom experienced by children with terminal cancer,[29,62] yet little is known about the pathogenesis or treatment of fatigue in this population. In all likelihood the etiology of fatigue is multifaceted, including factors related to the underlying disease; treatment of the disease; factors associated with intercurrent illness including anemia, infection, respiratory compromise, malnutrition, and cardiac, renal, and hepatic compromise; sleep disturbances; chronic pain; medication side effects; and psychosocial factors such as anxiety and depression. [63]

Thus, treatment of fatigue requires careful evaluation of the child aimed at identifying treatable underlying causes. In some situations, such as fatigue primarily related to advanced disease or organ failure, expectations for reversal of the symptom are limited. This should be explained to the patient and parents as a part of a plan to improve adaptation. However, some primary interventions pose relatively little burden for the patient. For example, the drug therapies administered to the patient should be reviewed whenever fatigue is prominent with specific attention to centrally acting drugs. The threshold for intervening to reduce physical inactivity, insomnia, some metabolic abnormalities, or depressed mood should be low. Exercise may be beneficial in relieving fatigue in cancer patients. [64] The sections that follow address treatment of other factors contributing to fatigue.

### Pain

#### *Incidence of Terminal Pain*

Most of the pain experienced by children with cancer occurs as a result of therapy rather than disease. [65] In the dying child, however, the pain most likely is related to advancing disease. Studies of the incidence of terminal pain in pediatric patients have provided insights about when pain may be anticipated to be a problem. More than 80% of children with advanced cancer experience pain, regardless of the underlying diagnosis. [29,66] However, patients with hematologic malignancy may experience pain for shorter duration in comparison to those with solid tumors. [66] If patients with solid tumors have impingement on the spine and major nerves, effective pain relief may require very aggressive measures, such as placement of an epidural catheter. [67]

#### *Barriers to Effective Pain Management*

To ensure effective pain management in children with advanced cancer, it is important to be familiar with common barriers leading to undertreatment of pain at the end of life.[68,69,70,71,72 and 73]

- Deficit in knowledge and experience can lead to undertreatment of pain. Examples of ineffective approaches include *pro re nata* dosing, using intramuscular administration of meperidine, or viewing starting doses of opiates as maximum doses.[74,75 and 76] In experience is especially prevalent among pediatric caregivers because deaths in childhood are infrequent.
- The unsubstantiated fear of inducing addiction can also impede the appropriate use of opioids for pain management at the end of life. [77,78,79,80 and 81] Parents of young children are particularly sensitive to this concern and at times need to have the difference between physical dependence and addiction carefully explained.[70] We avoid the term *narcotics* because of the connotations implied with its use, and instead use *opioids*.
- Further complicating opioids use is the symbolic meaning of opioids and the implication that beginning a “morphine drip” means giving up on the patient. [5,77] Again the emphasis here should be that maximizing comfort does not preclude continued hope and treatment of the underlying disease.
- What appears to be the most significant barrier among health care providers to administering adequate pain medication is the fear of hastening death through respiratory depression, excess sedation, or both. [82] However, there are virtually no empiric data in the literature that support the belief that appropriate use of opioids hastens death in patients dying from cancer or other causes. [83] In fact it is possible that the proper treatment of pain may prolong rather than shorten life.[84] None the less, care givers continue to be concerned that the cardiopulmonary side effects of the medications used to relieve the pain and suffering of dying patients have the potential to hasten the patient's death. In these circumstances, an ethical justification for aggressive palliation is the *principle of double effect*.[83,85,86] This principle stems from Roman Catholic moral theology and states that an action with both a good and a bad effect is ethically permissible if the following conditions are met:
  a. The action itself must be morally good or at least indifferent.
  b. Only the good effect must be intended (even though the bad or secondary effect is foreseen).
  c. The good effect must not be achieved by way of the bad effect.
  d. The good effect must outweigh the bad result.

  Thus, the good effect (pain control) is intended, whereas the bad or secondary effect (hastening death) is foreseen but not intended. This doctrine has been used to justify an aggressive approach to pain and comfort, even to the point of using barbiturates with the goal of inducing terminal sedation, when appropriate.[87]

Open communication regarding these issues among medical caregivers and the family may be an important means of overcoming these barriers.

### *Assessing Pediatric Pain*

The reliable assessment of children's pain is both a science and an art. It requires an understanding of child development so that the practitioner can choose an appropriate tool with which to undertake the assessment.[88] In addition to choosing the best assessment tool, it is most important for the caregiver to use that tool consistently. It has been shown repeatedly that there is poor correlation of pain perception between patients and caregivers in the absence of regular assessment. [89] Many have argued that pain assessment should be integrated as a fifth vital sign; that is, pain should be assessed on a regular basis for all patients, independent of their clinical status.[90]

### *Pain Management*

A comprehensive discussion of pain control in the cancer patient is found in Chapter 43. The basic approach to the terminally ill child in pain uses the World Health Organization Analgesic Ladder Program.[91] This is a stepwise approach to escalating therapy from weak analgesics [e.g., acetaminophen or nonsteroidal antiinflammatory drugs (NSAIDs)] to strong ones (e.g., morphine, fentanyl, or hydromorphone). Importantly, however, a multimodal approach should be considered in which medications, cognitive interventions, local anesthetics, and other pain therapies are used from the onset to limit pain perception and the pathologic responses to pain.[92] Practitioners should also have access to pediatric pain or palliative care services to help relieve the symptoms of the small group of patients who do not benefit from the standard approach.[2]

### *Pain Management: Setting the Stage*

Before the initiation of therapy for pain, it is worthwhile to clarify the meaning of pain with the patient and to establish mutual goals of therapy. There may be many factors that influence the child's perception of pain in addition to the physical damage initiating that pain. [93,94] The practitioner should assess whether the family's cultural, religious, or medical interpretation of the pain will have an effect on the treatment plan.

In addition, the team should attempt to help the patient and family devise reasonable therapeutic goals. Although ideally pain can be eliminated using nonpharmacologic approaches, often these are more effectively used as adjuvant rather than primary therapy. If medication is used to control pain, there are frequently side effects in addition to pain relief. [95] The goal from the standpoint of both the medical team and the patient and family is to obtain pain relief with neither euphoria nor sedation, and with effective control of any other side effects. If this goal proves to be impossible in a given patient's situation, the family and patient need to know that their decision about what is an acceptable toxicity profile will be respected by the team.

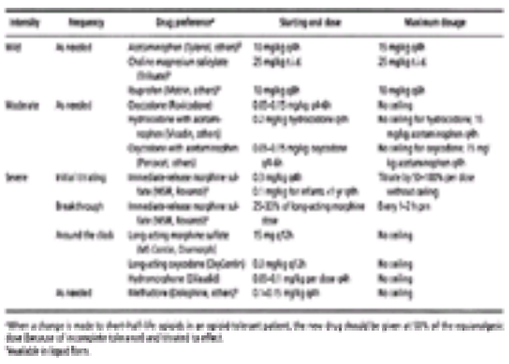
Families need to be educated about pain management. They need to know that opioid administration titrated against pain does not hasten death, that it does not lose its effectiveness over time, and that the dose can be increased as needed without a fixed ceiling. They should be taught that pain medications work most effectively when given around the clock so that pain does not have to be experienced to be relieved, that opioid administration rarely causes addiction in the cancer patient with pain, and that side effects most often can be managed without an interruption in pain relief. Patients and families need to know that they have access to medical personnel 24 hours a day for help with control of pain or other symptoms. Finally, they need to be informed that the simplest and most palatable means will be used to keep the child pain free.

### *Pharmacologic Treatment of Pain*

Some basic principles in treating pain in children with advanced cancer are as follows.

- The choice of a specific opioid agent should be directed primarily by the child's previous opioid experience.
- Keep the approach to pain management simple and consistent. The oral or buccal route of drug administration should be used in almost all cases, with rectal, transdermal, and systemic routes reserved for situations in which the caregiver is unable to reliably administer oral medication or pain escalation is so rapid that the oral route is not providing quick enough relief. Note that the rectal route of administration should be reserved for patients who are not neutropenic, unless there are no other options.
- Work with the patient and family to choose one drug based on pain assessment, and increase the dose of that drug as necessary until an unacceptable toxicity is reached. This is easier for the caregivers than multiple medication shifts, it is the most cost-effective way of treating pain, and it allows the care team to become very familiar the therapeutic effect and toxicity profiles of a limited number of drugs.

For mild pain, acetaminophen is the drug of choice (Table 51-2). If the pain originates from bony metastases, an NSAID should be considered.[96] Although ibuprofen has been found to be safe in normal children, many terminally ill children have compromised platelet numbers, so their function becomes critical. [97] Many of the effects on platelet functioning, renal blood flow, and the gastrointestinal (GI) tract may be eliminated with the introduction of NSAIDs, such as rofecoxib, that selectively inhibit COX II without effects on COX I, the enzyme present in the GI tract, renal system, and platelets.[98] Further investigation of these agents in the pediatric population is needed.



| Intensity | Frequency | Drug preference* | Starting oral dose | Maximum dosage |
|---|---|---|---|---|
| Mild | As needed | Acetaminophen (Tylenol, others)† | 10 mg/kg q4h | 15 mg/kg q4h |
| | | Choline magnesium salicylate (Trilisate)† | 25 mg/kg t.i.d. | 25 mg/kg t.i.d. |
| | | Ibuprofen (Motrin, others)† | 10 mg/kg q8h | 10 mg/kg q8h |
| Moderate | As needed | Oxycodone (Roxicodone) | 0.05–0.15 mg/kg q4–6h | No ceiling |
| | | Hydrocodone with acetaminophen (Vicodin, others) | 0.2 mg/kg hydrocodone q4h | No ceiling for hydrocodone; 15 mg/kg acetaminophen q4h |
| | | Oxycodone with acetaminophen (Percocet, others) | 0.05–0.15 mg/kg oxycodone q4–6h | No ceiling for oxycodone; 15 mg/kg acetaminophen q4h |
| Severe | Initial titrating | Immediate-release morphine sulfate (MSIR, Roxanol)† | 0.3 mg/kg q4h<br>0.1 mg/kg for infants <1 y q4h | Titrate by 50–100% per dose without ceiling |
| | Breakthrough | Immediate-release morphine sulfate (MSIR, Roxanol)† | 25–33% of long-acting morphine dose | Every 1–2 h prn |
| | Around the clock | Long-acting morphine sulfate (MS Contin, Oramorph) | 15 mg q12h | No ceiling |
| | | Long-acting oxycodone (OxyContin) | 0.3 mg/kg q12h | No ceiling |
| | | Hydromorphone (Dilaudid) | 0.03–0.1 mg/kg per dose q4h | No ceiling |
| | As needed | Methadone (Dolophine, others)† | 0.1–0.15 mg/kg q6h | No ceiling |

*When a change is made to short-half-life opioids in an opioid-tolerant patient, the new drug should be given at 50% of the equianalgesic dose (because of incomplete cross-tolerance) and titrated to effect.
†Available in liquid form.

**TABLE 51-2. PAIN MANAGEMENT GUIDELINES**

For moderate pain, oxycodone is the first-choice drug in treatment. It is available in concentrated liquid form. Because there is no ceiling dose, this drug could conceivably be used to carry a patient through the entire course of the illness. [99] Many practitioners discourage the use of codeine. The toxic effects of nausea, vomiting, and constipation with codeine seem higher than with oxycodone or hydrocodone. [99]

For control of chronic, severe pain in cancer patients, the use of immediate-release and long-acting morphine is advocated. In most situations, therapy begins with liquid or oral immediate-release morphine, allowing patient-controlled titration of the dosing for 24 to 48 hours. During this time, the caregiver should be in constant consultation with the family to monitor the need to increase or decrease the dose or timing interval. After the patient's opiate needs are established, the dose is converted to long-acting morphine (if the opiate requirement is high enough to be able to use the lowest-concentration dose). Methadone is an alternative long-acting opiate medication that is available in a liquid formulation. In these circumstances, methadone is most safely administered on an as-needed basis to mediate the effects of its long and unpredictable half-life. [98]

After the patient is receiving long-acting morphine, the initial titrating solution is used as a breakthrough medication. This morphine solution is made as concentrated as is feasible for the individual patient (usually 20 mg per mL) so that it may be given buccally if the patient is unable to swallow pills in the last days of life. Generally a rescue dose should equal 50% to 200% of the hourly dose or approximately 5% to 10% of the daily opioid requirements. [100] With rapidly progressive disease in the terminal phase, opioids may reach surprisingly large and well-tolerated doses. [67] Opioid titration for these opioid-tolerant patients must be made in significant increments, such as increases of 30% to 50%.[100] In addition, it is important not only to increase the continuous dose but to increase proportionally the breakthrough or rescue dose.

Other options are available when oral administration is either unacceptable to the child or precluded by physical conditions. Fentanyl is available via transdermal delivery through a skin patch. The patient must have relatively stable opiate needs, however, because this transdermal application takes 12 to 16 hours to reach effect once a change is made and therefore does not lend itself to frequent dose titration changes.

Once pain management is initiated, it is critical *to monitor the patient closely for the development of treatment-related side effects*. Caregivers should aggressively treat, or prevent, the development of the more common side effects of drug therapy. There are three side effects that often occur in the first days after therapy is started. They are usually temporary (48 to 72 hours) but may persist and be problematic. The first of these is sedation and somnolence. This side effect can be exacerbated if the patient was experiencing sleep deprivation before the initiation of opioid therapy. There is often a period of “catch up” sleep once a patient is comfortable. No treatment for this condition is needed except education and reassurance. If the symptom is persistent, however, both dextroamphetamine and methylphenidate have been shown to be effective in pediatric cancer patients in relieving unacceptable levels of somnolence. [101] Second, approximately 10% of patients show some manifestation of histamine release (e.g., flushing, itching, rash, nausea). This does not progress to anaphylaxis nor does it constitute an allergic reaction. These symptoms can be effectively treated with diphenhydramine, administered as needed or around the clock, depending on the severity of the symptoms. Nausea and vomiting can be another troublesome immediate toxicity associated with institution of opioid therapy. This symptom should be treated aggressively but not prophylactically unless a patient is already experiencing GI symptomatology. Hydroxyzine or a phenothiazine may be used to relieve the GI symptoms and should be initiated before the development of aversion to the opioid therapy.

Constipation is a chronic, persistent side effect of opioid therapy for most patients. It is one that is best treated prophylactically, usually with sodium docusate or senna therapy. These medications may need to be supplemented with other treatment regimens to ensure regular bowel movements. The dose should be increased concomitantly with increases in the opioid dose.

At high doses of opioid therapy, myoclonus can be a disturbing side effect. [102] This symptom should be treated vigorously by attempts to lower the opioid dose if possible or by use of lorazepam or clonazepam drug therapy. Persistent myoclonus or myoclonus progressing to seizures are reasons to change to another class of opioids for pain relief.

Although respiratory depression is extremely rare among patients receiving chronic opioid therapy for cancer pain, it is nonetheless a constant concern for the physicians and families as the patient approaches death. [82] If the physician or family is concerned that the opioid drug therapy could be contributing to the patient's decreased responsiveness or lowered respirations, attempts can made to lower the opioid dose. It should not be abruptly discontinued because of the possibility of precipitating physiologic withdrawal symptoms. This therapeutic trial usually shows that the pain returns rapidly and that it is the patient's overall condition that is deteriorating. Administration of an opioid antagonist, such as naloxone, can cause extreme distress and is almost never required.

### *Adjuvant Drug Therapies*

A number of adjuvant therapies have been shown to be clinically effective for treatment of pain in children dying of cancer, particularly in the following situations.

#### Bony Metastases

Bony metastases should prompt the initiation of therapy with one of the NSAIDs, such as ibuprofen. Bisphosphonates, such as pamidronate, have also been shown to decrease the experience of pain in adult patients with bony metastases, but this strategy has not yet been investigated in children. [103,104]

#### Neuropathic Pain

Neuropathic pain is caused by compression or infiltration of neural tissue by tumor. This type of pain can be notoriously difficult to treat and is frequently resistant to opioids.[105] These patients require different classes of drugs, such as tricyclic antidepressants and anticonvulsants, to control pain. [106] A child with pain and insomnia would do well with a sedating agent, such as amitriptyline. [92] Children bothered by anticholinergic side effects, such as sedation and dry mouth, can be treated with desipramine (which is relatively stimulating) or nortriptyline (which is less activating). A new anticonvulsant, gabapentin, is being used with increasing frequency in patients with neuropathic pain.[107,108] The principal side effect is sedation, which requires slow increase to therapeutic doses. This agent is not available as a liquid; however, children can chew the capsules.[92] If neuropathic pain is refractory to these strategies, invasive techniques, such as epidural or intrathecal catheters and neurolytic nerve blocks, may be required and must be considered early rather than as a preterminal therapy. [109]

#### Corticosteroids

Corticosteroids can have dramatically beneficial effects, but the adverse effects may be severe and occasionally devastating. Pain secondary to visceral distention, bony destruction, or cerebral edema can be mitigated with the use of steroids, either prednisone or dexamethasone. [110] Other positive effects can include appetite stimulation, combating nausea, and promoting euphoria.[92] Alternatively, these medications can lead to severe cushingoid appearance, hypertension, and glucose intolerance and can cause dysphoria. Thus, the use of corticosteroids must be carefully considered, and review of the child's past experience with these medications

can be helpful in deciding whether they should be initiated.

### Adjuvant Nondrug Therapies

There are a multitude of nonpharmacologic strategies, including guided imagery, hypnosis, meditation, acupuncture, and acupressure. [111] Very often, the pediatric oncology patient has used one or more of these techniques effectively to cope with the side effects of therapy or procedure-associated pain. This mastery allows the child to apply these methods to help control symptoms associated with the terminal phase.

Involvement of a therapist skilled in nonverbal communication can also be very helpful. This person may be trained in child life therapy or in the use of music, art, or movement therapy.[112,113] and [114] Therapeutic touch is currently under investigation as an adjuvant approach to pain management in patients with advanced cancer. [115,116] This type of therapy can be taught to parents of terminally ill children. Involving the parent in administering therapeutic touch can be a very meaningful experience for the entire family.[117]

### Depression and Anxiety

Psychological distress often causes suffering in terminally ill adults and their families and poses challenges in diagnosis and treatment. [118] Preliminary data suggest that depression may also be prevalent among children with cancer.[29] Diagnosing and treating depression in terminally ill patients involve unique challenges. Evidence of hopelessness, helplessness, worthlessness, guilt, and suicidal ideation may be better indicators of depression in this context than are neurovegetative symptoms. Chochinov and colleagues[119] found that simply asking, Are you depressed? was the best screening tool among adult patients. However, it is uncertain whether this approach would be useful for children.

Caregivers should have a low threshold for treating depression in children with advanced cancer. Psychological interventions—including eliciting concerns and conveying the potential for connection, meaning and reconciliation, and closure in the dying process—can facilitate coping. Psychostimulants, selective serotonin reuptake inhibitors, and tricyclic antidepressants are the mainstay of treatment for depression in terminally ill patients. They are particularly useful for patients who are seriously ill and may be unable to engage in psychotherapy. Psychostimulants (dextroamphetamine, methylphenidate, and pemoline) deserve special consideration in treating depression near the end of life, because they take effect quickly.

Anxiety may be a complicating factor in the pain management of children with cancer. In situations in which the anxiety is interfering with pain relief, lorazepam may be used as adjunctive therapy. Although there is a long-standing debate about whether the benzodiazepines possess analgesic property, they frequently have a positive effect on a patient's mood and level of anxiety.[120] Benzodiazepines are best limited to short-term or intermittent use; prolonged administration may lead to a decline in anxiolytic effect and cumulative psychomotor impairment.[121]

### Anemia and Bleeding

In children with hematologic malignancies or solid tumors metastatic to bone marrow, anemia and thrombocytopenia often occur in the terminal phases. The medical team should have a candid discussion with the family about how to handle symptoms associated with underlying marrow failure.

#### *Anemia*

If the patient demonstrates symptoms from anemia (e.g., decreased strength, dizziness, shortness of breath, tachycardia) or has signs of continued blood loss, periodic transfusion of red blood cells may be an appropriate palliative course of action. [122] Laboratory investigation should be kept to a minimum, with the team oncentrating instead on evaluation of the symptoms and the response to transfusion. There should be no assumption that transfusions will be continued after they are started, because the clinical situation may change, and there may come a time when further transfusions are unlikely to benefit the patient. The role of erythropoietin and impact on quality of life in terminally ill children with cancer is largely unexplored. [123]

#### *Thrombocytopenia*

If the child is anticipated to be thrombocytopenic and begins to manifest bleeding symptoms (e.g., nosebleeds, GI oozing), then the possibility of platelet transfusion support should be discussed with the family.[124] Generally, the child must travel to a medical center for this transfusion, and because platelets are more short lived, this is a more intrusive form of palliative support. Massive external bleeding is unusual in the terminal pediatric cancer patient, so the medical team should feel comfortable in supporting whatever approach is desired by the patient and family.

### Central Nervous System Symptoms

#### *Seizures*

Seizures can be a very distressing symptom for patients and families when they occur during the terminal phase. Although most common in the presence of brain tumors or metastases of other cancers to the brain, they sometimes occur spontaneously with central nervous system bleeding because of low platelet count or hypocoagulability. For new onset of seizures, in whom discovery of an intracranial mass may result in a course of radiation therapy for palliation, it may be reasonable to investigate the cause through imaging.

If seizures occur in a patient who is taking anticonvulsant therapy, they can often be controlled by increasing the dose of the medications already prescribed. If this is inappropriate because of problems with the route of administration or the long half-life of the drugs the patient is taking, a short-acting benzodiazepine can be used to suppress the seizures quickly. Some hospices use the intravenous (i.v.) solution of diazepam, which can be administered rectally with the use of a special bulb syringe. An alternative is to use lorazepam, which can be administered buccally to the seizing child. If the child is at risk for seizures it is highly recommended to have a benzodiazepine readily available.

#### *Increased Intracranial Pressure*

Increased intracranial pressure is sometimes problematic in children who are dying as a result of a brain tumor. If maximum radiation doses have previously been administered, as is frequently the case, the main therapeutic option left is to increase the dose of dexamethasone. It is important to involve the patient and family in the decision to increase steroid doses to control symptoms of headache, nausea, vomiting, and increased somnolence. The side effects of long-term dexamethasone therapy, including weight gain and the development of a cushingoid appearance, can be so disturbing to some children that they place limits on the amount of drug that they are willing to take on a chronic basis. There are no data regarding the risks and benefits of shunt placement in the setting of increased intracranial pressure for children with advanced cancer. The tradeoffs between the morbidity of the procedure and possible later complications, and the potential benefit of relieving pressure need to be carefully considered.

#### *Spinal Cord Compression*

Spinal cord compression resulting from epidural metastases, although uncommon, can result in significant morbidity in the child with advanced cancer. Thus, new onset back pain should be carefully evaluated. Normal findings on physical examination do not diminish the probability of cord compression, and magnetic resonance imaging is the preferred evaluation technique. If a patient is treated while he or she is still ambulatory, the probability of remaining ambulatory is 89% to 94%. [125] Even ifthe patient loses some function, earlier intervention can lead to improved outcomes. Corticosteroid therapy decreases cord edema and pain, helps preserve neurological function, and improves overall outcome after specific therapy. [126] Radiation therapy may also be helpful for radiosensitive tumors.

### Fever and Infections

When assessing the terminal child with fever to determine whether a diagnostic workup should be attempted or antibiotic therapy begun (or both), it is most important to focus on the current goals of the patient and family. The approach can range from a complete workup with i.v. antibiotic administration to empiric treatment of

infections contributing to the child's discomfort, such as dysuria with frequency due to a urinary tract infection. Similarly, if a child should develop a fever, cough, and increased respirations, many palliative care teams would assume that aspiration or pneumonia has developed without requiring chest x-ray films for confirmation. After many months of constant vigilance against infection while the child was on chemotherapy, however, it may be difficult for families to watch the development of fever in the child and not take the typical approach. It is therefore critical to carefully explain the options to families and determine together what is best for the child. Factors to consider include how responsive the infection may be to antibiotics, whether they can be administered in the patient's setting of choice, whether there is significant toxicity from the antibiotics or their administration, and how uncomfortable the child may be were they to be withheld. [127]

Many hospices choose to treat relatively straightforward infections, such as pneumonia, urinary tract infections, and skin infections, with oral antibiotics in the home. More invasive infections such as sepsis or widespread fungal disease may be difficult to control without significant toxicity to the child. It is important to explain to families that death resulting from sepsis can be very peaceful, and in certain circumstances, intervening with i.v. antibiotics may only serve to prolong suffering.

The discomfort of fever can usually be controlled with acetaminophen alone or with acetaminophen combined with ibuprofen. Environmental manipulation will also help keep the child comfortable.

### Gastrointestinal Symptoms

#### *Nutrition and Hydration*

Nutrition and hydration in the terminal child are complex issues evoking intense emotional response in medical caregivers and families. The cancer anorexia-cachexia syndrome is extremely common in children with advanced cancer and is frequently associated with a patient's decline and death. [29,128] Its cause is multifactorial, and it is most often irreversible, even in the face of hyperalimentation or vigorous nutritional support. [129]

The use of supplemental hydration and nutrition in children with advanced cancer is controversial. [130] Some have argued that the naturally occurring decreased oral intake is not associated with symptoms of hunger and thirst in most patients. [131,132 and 133] thers contend that dehydration may contribute to patients suffering. [134] Family members often find these symptoms most distressing.[135] Clinical studies do suggest that terminally ill cancer patients may achieve adequate hydration with much lower volumes than recommended for the average patient.[136] For these reasons, it is important to educate families about the normalcy of decreased appetite and thirst in the dying child. The goal of nutrition and fluid management should be to alleviate any hunger and thirst, to reduce anxiety, and to preserve the social aspects of mealtimes.[137] Patients may find consumption of small, frequent meals more manageable than large meals. Magestrol acetate can be used to stimulate appetite and promote weight gain in patients with advanced cancer with little concern for toxic side effects. [138,139] The use of more invasive strategies such as gastrostomy tubes, or i.v. hydration or nutrition should be carefully considered in light of individual patient and family needs.

#### *Nausea and Vomiting*

Nausea and vomiting can be a problem in the dying child, either as a result of tumor invasion or as a consequence of opioid therapy. It is helpful to attempt to establish the cause of the nausea before treatment.[140] In addition to reviewing the medications of the patient and signs of abdominal tumor involvement, it is important to rule out impaction from chronic constipation. As previously discussed, nausea resulting from increased intracranial pressure may be alleviated with dexamethasone. There are many pharmacologic approaches to the treatment of nausea and vomiting in children, and the practitioner must make a decision based on the patient's previous experience with antiemetics and an evaluation of the current problem. [141] Selective 5-hydroxytryptamine antagonists have been found to be effective for patients with advanced cancer, whether or not they are receiving chemotherapy. [142,143] Phenothiazines should be used with caution because of concern for extrapyramidal side effects, and should be administered with concomitant diphenhydramine. The addition of dexamethasone and lorazepam (Ativan) should be considered with refractory symptoms.

#### *Constipation*

Knowledge of the underlying cause of constipation helps in both prophylaxis and treatment. The most important of these are immobility, poor fluid and dietary intake, and drugs, particularly opioids. [144] Less commonly, GI obstruction or neurological compromise results in constipation. Effective management of constipation starts with a careful assessment of the patient, including history of the frequency and difficulty of defecation. When the diagnosis of obstruction is unclear, an abdominal x-ray may be required.[145] The management of constipation extends well beyond the use of laxatives. Attention to other symptoms, especially pain, and advice on diet, fluid intake, mobility, and other activities of daily living contribute to an effective outcome. As soon as a patient is begun on opioids, a bowel regimen should be instituted. Specifically, a softener such as sodium docusate or lactulose should be used in combination with a stimulant such as senna. Rectal laxatives may be necessary to treat severe constipation or impaction; however, they should not be part of regular treatment. They are undignified and may have a considerable negative effect on quality of life.

#### *Intestinal Obstruction*

While surgery remains the primary treatment for malignant intestinal obstruction, some patients with advanced cancer or poor general condition are unfit for surgery and require alternative management to relieve distressing symptoms. Many of the symptoms of GI obstruction can be relieved with pharmacologic methods, including the combination of morphine, scopolamine, and haloperidol. [140] Corticosteroids have also been useful for patients with intestinal obstruction. [146]

### Dyspnea

Dyspnea has been defined as an “uncomfortable awareness of breathing.” Dyspnea is a common symptom among children with advanced cancer and can result in substantial suffering.[29,62,147] It is important for the team to consider the cause of the respiratory distress and adopt the most efficacious treatment. For example, dyspnea resulting from pneumonia may be effectively treated with an oral antibiotic regimen. Congestive heart failure is, in general, unusual in pediatric cancer patients, but there are times when cardiomyopathy or chemotherapy cardiac toxicity is a significant problem. [148] Drug therapy includingan angiotensin-converting enzyme inhibitor or diuretic may be beneficial. Drainage of even small quantities of fluid can greatly relieve dyspnea resulting from pleural effusion; however, rapid reaccumulation of fluid is common, and the relief from thoracentesis may be quite temporary. More invasive approaches to pleural fluid, including chest tube placement and instillation of sclerosant drugs, should be carefully considered. In very weak patients with a short life expectancy, the discomforts resulting from this approach may outweigh any benefit in control of symptoms.[149]

The most common cause of respiratory distress in pediatric cancer patients is pulmonary metastases that interfere with oxygen exchange. Important to the success of managing this symptom is routine systematic assessment. Many have adapted a visual analog scale to assess the discomfort arising from dyspnea, although this approach has not yet been validated in children. [150] Most studies have found that systemic opioids of different types are effective in treating dyspnea by relieving feelings of suffocation. [151] Supplemental administration of as little as 25% of the equivalent 4-hour dose can provide substantial relief. [152] Nebulized opioids have not proved to be beneficial thus far. [153] Supplemental oxygen can also have a very beneficial effect on patients with cancer experiencing dyspnea even when it is unrelated to hypoxia.[154] However, it is important to consider the pros and cons of oxygen therapy on a case by case basis. [155] The use of a nasal cannula instead of a mask can be more palatable. The gas can be humidified, but this can be noisy. A 24-hour trial of continuous or intermittent oxygen therapy may be appropriate. Finally, benzodiazepines have a place in managing dyspnea even in patients who do not have prominent anxiety. [155]

## MEANINGFULNESS AND QUALITY OF LIFE AT THE END OF LIFE

Adequate pain and symptom management, strengthening relationships with loved ones, and avoiding inappropriate prolongation of dying are among a set of priorities elicited from adult patients with terminal illness.[156] Similar research has not be conducted in children or their parents. However, experience teaches us that these are critical considerations. Furthermore, families must have the opportunity to carry out important family, religious, or cultural rituals during the child's end-of-life care period.[157] The families' sense of spirituality or engagement in a religious community may provide a structure for positive coping strategies for both parent and child. [158] The goal is to add life to the child's years, not simply years to the child's life. [2] Facilitating memory building during this period can be the greatest gift to the child and family.

For many children, the social context of school and friendship is most important. The care team should encourage the child's continued participation in a school

setting, even if attendance is limited by the child's physical deterioration to “social” visits. Whether the child is based at home or in an institution, regular social contact with other children and adults should be strongly encouraged. This may involve a shift of attitude in families that have been very protective about visitors for fear of introducing infection to the child on chemotherapy.

## WHEN DEATH IS IMMINENT

During the last few days of life, patients experience increasing weakness and immobility, loss of interest in food and drink, difficulty swallowing, and drowsiness. [159] This phase usually can be anticipated, but sometimes a deterioration can be sudden and distressing. Control of symptoms and family support take priority, and the nature of the primary illness becomes less important. This is a time when levels of anxiety, stress, and emotion can be high for patients, families, and other caregivers.

There are several key principles in managing the child's final days. An analytical approach to symptom control continues but usually relies on clinical findings rather than investigation. Drugs should be reviewed with regard to need and route of administration. Some patients manage to take oral drugs until near to death, but many require an alternative route. Finally, it is essential that the care team maintains effective communication during this time and ensures that support is in place for the family. A daily visit for inpatients or a daily phone call at a planned time can be very reassuring for families.

Importantly, even when the child may be comfortable and symptoms well controlled, simply being a presence during the final period can be very comforting to family members. Such a presence reinforces that the dying patient's welfare remains important, and it provides support and guidance to the family at a time of extreme stress. It is critical to inform the family that although death may be imminent, the time frame may be hours to days. Medication should be escalated in response to the child's suffering and not the suffering of the family. It is essential to ensure that someone will be available to pronounce the child, especially when the child is not in the hospital.

### Terminal Sedation

The need for sedation arises in rare situations, either because a patient's pain cannot be controlled despite all efforts or because of the development of terminal restlessness and agitation. It is critical to be clear and honest in describing to the family and to other caregivers how terminal sedation will effect the child. The usual approach is to increase the dose of the opioid for the child already being treated with opioids. In the circumstance of significant opioid tolerance and a very distressing symptom, however, sedation with opioids alone may be ineffective, or unwanted side effects may be exacerbated with opioid escalation. [160] This warrants the inclusion of a second agent, such as a neuroleptic, benzodiazepine, or barbiturate, while continuing opioid therapy. [87,161] Rapid titration to the end point of comfort through sedation may be required for rapidly progressive symptoms, as sometimes witnessed in the imminently terminal phase. Importantly, in this setting, medication should not be administered via i.v. push. This can lead to the last dose of a medication being associated with the child's demise. Rather, medication should be administered via continuous infusion with dose escalation by 50% as needed. Extreme vigilance for breakthrough symptoms or adverse effects is warranted.

### Death Rattle

Breathing can become particularly noisy when death is imminent, often described as the *death rattle*. This is more common in patients with primary lung disease or brain tumors.[162] It is critically important to prepare family members for this possibility. Because this symptom is often present when the child is already unconscious, the child may not experience this as uncomfortable. However, transdermal scopolamine or L-hyoscyamine drops for smaller patients can be helpful in drying secretions and diminishing this symptom.[163]

### Autopsy

When a child dies from progressive cancer, medical caregivers may feel that there is no reason to perform an autopsy. However, postmortem examinations may provide a great deal of additional information. Sirkia and colleagues [164] found that in 40 children who died of progressive cancer autopsy examinations afforded totally new information in 20% of cases and important additional information in 55%. In addition, whether or not new information is uncovered, families report that knowing the findings at autopsy is helpful for them, and the vast majority who consent believe that autopsy of their child would at least be helpful to other patients. Furthermore, the autopsy provides an opportunity for families to return for a follow-up discussion, often an important step in bereavement.

Importantly, the decision to perform an autopsy should not be influenced by the place of death. In the experience of the Midwest Children's Cancer Center at Milwaukee Children's Hospital, home care did not reduce the incidence of postmortem examinations for research purposes. In their series, autopsies were performed on 57% of the children who died at home, compared with 47% of cancer-related hospital deaths. [165]

In order for this important decision to be fully considered, families should be given the opportunity to consider this request before the child dies. Given the sensitive nature of this request, it is best discussed with a member of the primary care team.

## BEREAVEMENT

One of the most important tasks of the care team is to provide bereavement support for the patient's parents and siblings. The death of a child has a profound and lasting impact on the family unit.[166] During this long period of mourning and reorganization, parents and siblings can be supported in several ways.

- At some interval after the child's death, review with the family the medical events surrounding the illness and terminal phase. If a postmortem examination has been performed, the results can be included in this discussion. Parents often have significant medical questions that need to be answered before the psychological work of mourning can take place. Ideally, a physician or nurse familiar with the case should initiate this contact so that the questions and concerns can be answered specifically. Siblings of a dying child often hold misconceptions and misunderstandings that cause confusion in the weeks and months after the death. Specific, concrete information about the deceased child's illness as well as the siblings' own health may do much to allay fears. [167]
- Offer educational materials about the process of grief and mourning. Anticipate such challenging times as the first holiday season, the first birthday, and the first anniversary of the death. There are many resources written for adults about the grieving process. [167] In addition, many children's books on dying, death, and bereavement are available for families to use in helping siblings mourn ( Table 51-3).

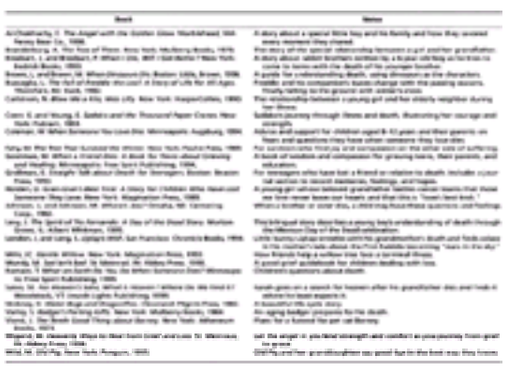


**TABLE 51-3. LIFE CYCLE STORIES FOR CHILDREN**

- Identify abnormal patterns of grief within the family. The bereavement practitioner should be cognizant of high-risk mourning situations in both parents and children so that pathologic grief may be recognized.
- Invite bereaved parents and siblings to receive support from others. There are a variety of resources for the family of a deceased child. These include special interest groups, such as Compassionate Friends, as well as the bereavement groups of hospices and hospitals. The bereavement specialist should also be aware of therapists in the geographic area who can provide expert counseling to those who would prefer individual consideration. In addition to time-limited or ongoing children's bereavement groups, some hospices and hospitals also offer a camp experience for bereaved children.
- Establish memorial rituals for families of deceased children. These can take place either in the context of the tertiary medical center or in the community. They

provide both a powerful reaffirmation of the importance of the deceased child and a time when parents and siblings can reunite with those who cared for their child.

- Be prepared to follow bereaved families for a long time. The death of a child is so shockingly abnormal that the parents' bereavement period often extends for months, if not years, longer than is usual for other, more anticipated deaths. [168] In addition, as siblings grow through different developmental phases, they will probably find it helpful to reprocess the death in light of their newfound knowledge and emotional capabilities. For this reason, it is important for the bereavement counselor to remain available for long periods after a child has died.

Although it can be said that families never “get over” the death of a child, in most cases they are able to accomplish the tasks of mourning and find meaning and purpose in life once more. It is the task and commitment of the care team to stand with the family, ready to give support if needed throughout their work of grief and mourning.

## CARE OF THE CAREGIVERS

The care of terminally ill children and their families is extremely rewarding. Nonetheless, the repeated losses experienced by medical caregivers may constitute a significant source of personal stress. [169] Studies of physicians have documented high prevalence of alcoholism, cirrhosis, suicide, and marital discord. Significant etiologic factors include death as an existential fact emphasizing our finite nature, the cumulative grief associated with repeated unresolved losses, the pressure of a health care system fueled by the medical information explosion, the inability to achieve the idealistic goals embraced by holistic medical care, stresses inherent in working as a “team”; and an undermined context of meaning as an outcome of treatment failures.

Strategies useful in the prevention and management of stress include the encouragement of increased awareness of stress in self and colleagues, the clarification of appropriate goals and priorities, encouragement of appropriate limit setting, the clarification of team roles and organizational patterns, the establishment of team support meetings and favorable working conditions, exercise, and the clarification and working through of previously unresolved personal psychodynamic issues.

## ONGOING CHALLENGES IN THE CARE OF THE DYING CHILD

Optimal care of the dying child requires the unified effort of an interdisciplinary team. Although the principles of pediatric palliative care have been defined and refined over the last two decades, notable challenges remain.

- The tertiary pediatric oncology center and the community agency must forge a respectful partnership in caring for dying children and their families. They should recognize and acknowledge one another's areas of expertise and allow the family to draw strength from both sources.
- The medical professional should advocate for governmental and legal support for a symptom-free death in an environment of the patient's choosing. Currently, there are many legal and health care reimbursement policy impediments to administration of optimal hospice home care. [170,171]
- The United States is currently facing an emotional public discussion on the question of assisted suicide and euthanasia. Although children have not been included in this debate, it is important for the practitioner to be cognizant of the issues and supportive of educational debates on the quality of death in this country.[172,173]
- The medical professional should work toward assuring that all dying children and their families receive the support of a trained interdisciplinary team. [2] They mustalso recognize the long-term needs of families and ensure the continuity of service through time. This requires creating a supportive environment for staff so that they are able to maintain a high level of commitment to the field and enjoyment and satisfaction in their work.
- Finally, the field of pediatric palliative care is in need of rigorous research efforts aimed at developing ways to enhance communication, symptom management, and quality of life for children with terminal illness. These efforts should be integrated into curricula addressing end-of-life care for medical and nursing students, residents, and faculty.[174]

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# FINANCIAL ISSUES IN PEDIATRIC CANCER

SUSAN K. PARSONS



## INTRODUCTION

Considerations of the current health care system have a Dickensian quality about them: the best and worst of times. Amid stunning innovations and advancements in diagnostics, therapeutics, and supportive care outlined elsewhere in this textbook is a fiscal reality that is far more grim. For more than two decades, societal allocations for health care in overall dollars and as a percentage of the gross domestic product have been growing—indeed, spirally in “crisis-level” proportions. The numbers are staggering. Overall, more than one trillion dollars will be spent in the year 2000, approximately 10% of which is for the cost of cancer. The annual cost of cancer, $107 billion, represents 3% to 4% of the gross domestic product. [1] Approximately one-third of the total cost of cancer is for direct medical costs ($37 billion or 34.6%), over half of which is tied to the treatment of breast, lung, and prostate cancer. [2] The treatment of colorectal malignancies contributes an additional $6 billion to this figure.[3] The remainder is attributable to mortality costs of $59 billion (lost productivity due to premature death) and morbidity costs of $11 billion (lost productivity due to illness).[2]

Although there is considerable debate about the forces responsible for this growth, part of it can be explained by technological and biopharmaceutical advances that have resulted in the enhanced ability to deliver increasingly resource-intensive treatments. Another force is increasing expectations to “do everything.” The fiscal consequences of a “no holds barred approach” are daunting.

Recent legislative and regulatory responses to unchecked growth have resulted in substantial budgetary restrictions on Medicaid and Medicare reimbursement. These cuts, coupled with increased managed care penetration, are having a stinging effect on the institutions providing care. The impact to other stakeholders is equally startling. Despite ever-increasing levels of spending, there is a growing number of medically uninsured, a pool overrepresented by children and young adults. Despite continued economic expansion and a 30-year low in the unemployment rate,[4] the proportion of privately insured people is dropping. Equally troubling is the declining willingness or ability of providers to deliver uncompensated or charity care. [5] This has been shown to be especially true among providers in areas of heavy managed care penetration and those involved with managed care plans. [6] The predominance ofcharity or uncompensated care has shifted to institutions that are already financially strapped (public, nonprofit, teaching hospitals). [5]

Faced with increasing fiscal pressures, including diminishing reimbursement and a shift in risk from payers to institutions, hospitals are searching for ways to decrease costs. One of the principal ways to decrease cost is to decrease hospital stays. Technological innovations as well as growth in the infrastructure of outpatient and home-based care have aided this. Changes in the locus of care have included complete shifts to the outpatient clinic or home as well as hybrids including early hospital discharge or delayed hospital admission. Increasingly complex, technologically intensive care is being provided outside of the hospital ward. The initial shifts to outpatient or home-based care were quite attractive financially for many institutions, particularly in the unbundling of care influenced by prospective payment. A classic example is outpatient testing and evaluation before elective hospitalization. Over time, however, with increased complexity of out-of-hospital care, these solutions have not been a financial panacea to either the institutions or the patients. For the institutions, inadequate reimbursement of outpatient services has resulted from reductions in payments from third-party payers and the institutions' historical decisions regarding the allocation of cost between inpatient and outpatient services.[7,8] For patients and their families, the shift away from hospital-based care has had three major effects. Decreased institutional reimbursement has resulted in more cost passed onto families, increasing out-of-pocket (OOP) expenditures for uninsured care. In addition, outpatient care has the potential for increased lost income for family caregivers because care is delivered during traditional working hours. Lastly, families incur the increased burden and responsibility of caring for the patient at home, a role for which families have varying financial, physical, and emotional capabilities.

The impacts on institutions and families resulting from the significant changes described above in the organization and reimbursement of care require careful analysis and evaluation. Although these issues are germane for the entire health care system, they are amplified in oncology due to rapid technological changes, extreme costs of treatment, and the burden of home-centered care given the severity of illness. Unfortunately, the existing analytic tools only partly address the wide range of issues, particularly in pediatric oncology.

This chapter opens with a discussion of techniques currently available to evaluate cost, including the progress and challenges in the application of these techniques to pediatric oncology. The second section focuses on the institutional perspective in the cost debate, including a review of the strategies that have been implemented in shifting the locus of care (and cost) and addresses the need to reengineer the relationship between cost and payment to reflect changing sites of care. The third section presents a discussion on the impact of the current fiscal situation on patients and families, including the need for a broader understanding of the role and the capacity of the caregiver in delivering care.

## METHODS OF ECONOMIC EVALUATION

Cost analysis provides a mechanism to formally review what is being spent, where, and by whom, as well as a description of the results or outcomes of those expenditures (in dollar terms or in the measured health effect). This type of analysis aids in identifying the gaps or the problems with current expenditures and helps delineate future trends. Clinical (economics) research with respect to cost analysis had its nascence in the late 1970s and 1980s, in response to continuing growth of health care expenditures and increasing regulatory involvement. In its current configuration, economic evaluation of health care is the comparative analysis of alternative programs of action with respect to both the costs and the consequences.

Economic evaluation of health care expenditures assumes that although demand for services is infinite, resources for health care are scarce. [9] This assumption is most readily recognized in those societies with a single-payer or centralized system. It assumes that unspent dollars (dollars saved) will be reallocated to other programs or services within and beyond health care (the opportunity cost). In the United States, in which there is a free-market system, the notions of scarcity of resources, competition, or rationing have not been historically endorsed, although they are becoming more widely recognized in responses to unbridled growth. Economic pressures on the health care system, influenced by the total amount of expenditures, rate of growth, and competition for dollars by other sectors of the economy, have

resulted in increasing attention to the process by which resources for health care are allocated. The cost of cancer care is under particular scrutiny due to a number of factors, including the use of new and expensive technologies, the potential for changing sites of care delivery, and the aging of the population. Many would argue that even if resources were unlimited, one would want to evaluate treatments to understand more fully the tradeoffs and to establish priorities. Economic analysis in health care results in the unusual wedding of principles of economics and clinical decision making—linking planning decisions on behalf of the community with clinical decisions made on behalf of the individual patient.

## Components of Cost

There are three major categories of cost: direct, indirect, and intangibles. Direct costs include medical and nonmedical costs. *Direct medical costs* include the costs of medical services, whereas *direct nonmedical costs* are the costs incurred in receiving medical care. *Indirect costs* are the costs of lost productivity related to the illness. *Intangibles* are the costs of pain and suffering.[10] The components of these costs and sources of data are summarized in Table 52-1.

| Category | Components | Sources of information |
|---|---|---|
| Direct medical | Hospital and clinical services | Administrative database (workload units or dollars) |
| | Professional fees (doctors and nurses) | Billing information |
| | Diagnostics (laboratory, radiology) | Insurance billing data |
| | Therapeutics (pharmaceuticals, transfusions, infusions) | |
| Direct nonmedical | Transportation | Patient/family cost diaries or estimates |
| | Relocation/geographic displacement | |
| | Alternate child care | |
| | Home modifications (equipment/supplies) | |
| | Out-of-pocket expenses (insurance premiums, deductibles, copayments) | |
| Indirect | Changes in wages (base and overtime pay) | Employment records |
| | Consumption of benefits | |
| | Change in employment status (job shift, loss, or total) | |
| Intangibles | — | Estimates based on willingness to pay |

**TABLE 52-1. COMPONENTS OF COST AND SOURCES OF COST DATA**

## Perspective of the Analysis

The inclusion of which costs and whose benefits is determined by the perspective or point of view of the analysis. The perspective can be the patient and/or family, the institution, the third-party payer, or society—depending on the study question. The societal perspective is framed by the economic pressures of the health care system and assumes that reduction in total resource use is desirable. The caveat is that the quality of care and patient outcomes are maintained.

Occasionally, it is beneficial to evaluate results from more than one perspective to determine whether they are the same. If they are not, the analysis helps to make the differences explicit.[11] For example, Eisenberg and Kitz[12] reported that early hospital discharge of patients with osteomyelitis was $510 less expensive (per episode) than continued inpatient care. From a societal perspective, early hospital discharge would appear to be the most cost-conscious strategy. If home care or oral antibiotics were not covered by insurance (and inpatient care was), however, the same analysis from the patient's perspective would render very different results. Concerns over decreased compliance, altered satisfaction, or erosion of quality of care may arise if the financial burden is too high (from the patient's perspective).

## Types of Analyses

Several types of analyses have been developed to incorporate these components ( Table 52-2).[11,13] They differ chiefly in the way in which the consequences or outputs of strategies are measured.[14]

| Type | Features |
|---|---|
| Cost accounting | Description of costs (typically direct medical costs) of a treatment. |
| Cost minimization | Comparison of costs of alternative strategies. Assumption that if clinical outcomes are identical, less expensive strategy is preferred. |
| Cost effectiveness | Comparison of incremental benefit (expressed in life-years saved) of one strategy versus another, incorporating additional costs of that strategy. |
| Cost utility | Extension of cost-effectiveness analysis with the added feature of introducing values (weights) to the health effect. Yields quality-adjusted life-years. |
| Cost benefit | Consequences (benefits) of intervention (program or treatment strategy) translated to monetary terms to yield net benefit in dollars. |

**TABLE 52-2. TYPES OF ECONOMIC ANALYSES**

*Cost accounting* relies only on the inputs or costs. This is a descriptive approach in which the costs of a strategy and the relationship between cost centers are derived. Its applications are principally in the areas of cost allocation, identification of cost drivers, and program planning. The challenges of this method are the proper determination of all costs as well as addressing the potential distortion in charges versus actual costs.

*Cost minimization* assumes that if the clinical results are identical, the less expensive strategy is preferred. This approach augments the cost accounting method with comparison. In this method, also referred to as *cost identification*, the costs of one strategy are compared with the costs of another strategy. For example, if we assume that an oral agent works as well as an intravenous (i.v.) agent, the oral agent is preferred because it is less expensive. The challenges with this method include adequate information on clinical results based on the two strategies and a complete accounting of costs for each strategy. In the forgone example, it would be imperative to know how parity is determined—that is, for which regimens and with what group of patients (based on socioeconomic characteristics). In identifying the cost of the two drugs by route of administration, the drug cost, preparation cost, and nursing costs would be collected for each route. Cost identification analysis is useful in determining the financial burden of disease or cost of medical care. It does not, however, evaluate expenditures in terms of health effect. [11]

*Cost-effectiveness analysis* (CEA) evaluates the additional benefit of one treatment over another, incorporating the additional costs of that strategy to yield a ratio of dollars per life-years gained by that strategy. The lower the value of this ratio, the greater the benefits derived from this health expenditure. [15] One of the important differences between CEA and cost minimization analysis is that the alternative strategies in CEA do not necessarily yield the same clinical results. Two key components of this analysis include appropriate discounting of future costs and health benefits to their present value and at the same rate. In addition, to deal with the uncertainty of outcomes, sensitivity analysis should be performed on key parameters. [16,17] The relative stability of the results strengthens the conclusions; alternatively, unstable results with the varying of selected parameters may inform areas of further research. Typically, programs or treatments are considered “cost effective” if their cost is less than $50,000 per life-year gained. Conversely, programs greater than $100,000 per life-year gained are not considered cost effective. Results in the middle range are “intermediate.”[18] These thresholds are not “absolute” but rather reflect willingness to pay a specific amount of money for this health benefit. If we are operating from a fixed pool of resources, the value of this health benefit must be weighed against the value of other health benefits—as well as against other programs or services in society (broadest sense of opportunity cost), even if the CEA ratio is “favorable.” [19]

*Cost utility analysis* is a variation on the methodology of CEA, incorporating the value of the health effect. Utility scores or weightings reflect preference measures of health-related quality of life. The utility weighting times the number of life-years gained produces the quality-adjusted life-years. [14] There is considerable debate about the source of the utility—specifically whether it should be assigned by representatives of the general population or informed by patient preference. This debate is further complicated in pediatrics in considering whether the patient should determine the weights or they should be set *on the patient's behalf* by proxy reporters.

Alternatives to the quality-adjusted life-years include disability-adjusted life-years, which include built-in age weights; healthy-years equivalents; and save-young-life equivalents. The theory and methodology for healthy-years equivalents and save-young-life equivalents are still under development. [20]

In *cost-benefit analysis* the consequences of a program of action are the monetary units, based on the conversion of benefits and all costs to dollars. The results of the analysis can be reported as a ratio of benefits to cost as well as a calculation of the net benefit. The latter is more informative from a societal perspective. In many ways this methodology is both the most appealing in its completeness and the most difficult to perform. Historically, this form of analysis has been used to evaluate projects or programs. Its application to health care decisions is far more precarious. Three major criticisms have been raised: the first is how to assign value to human life. That is, how do we monetize something that is not normally exchanged in the marketplace? Second, how do we estimate production gains, based on future earnings in a way that is equitable? If we base future earnings on current earnings or other proxies of societal position or employability, we penalize low-income groups, the elderly, and the handicapped. The application to children's projected earnings is even more nebulous. The third component is in determining the economic value of intangible benefits or intangible cost. One proposed method is “willingness to pay” to either alleviate suffering or avoid added risk. This method is imperfect in two ways: first, it is influenced consciously or unconsciously by the economic situation of the respondent—specifically, having money to spend enhances the likelihood of willingness to pay. Second, it assumes perfect knowledge, perfect mobility, and voluntary choice. Rarely do we have access to these three requisites. [9,21]

**Methodologic Standards**

To render cost analyses most informative in guiding clinical decision making and ultimately, in shaping policy, several researchers in this area have called for improved methodologic standards in performing and reporting results. The Panel on Cost-Effectiveness in Health and Medicine, convened by the U.S. Public Health Service from 1993 to 1995[16,17] is an example of such an effort; the recent comprehensive review by Earle and coworkers[22] of cost utility analysis is another. The proposed guidelines for conducting and evaluating economic studies are summarized in Table 52-3.[23,24,25] and [26]

| |
|---|
| The study question and design are clearly stated, relevant, and appropriate. |
| The setting, the study period, and patient characteristics should be clear to allow inference about similar results in other populations. |
| The perspective of the study is clearly stated. Although alternative perspectives may be relevant for specific study questions, in general, the societal perspective is preferred. |
| All costs and benefits should be identified, measured accurately, and valued credibly. Actual cost data versus charge data are preferred.[13] While typically the health effect is life-years gained/saved, other benefits include decreased side effects or morbidity, or increased quality of life, satisfaction, and productivity, among others.[26] |
| Future costs and benefits should be discounted (equally) to present value. |
| Sensitivity analysis should be conducted for key variables across a reasonable range of values. |
| Data should be presented in a transparent fashion. |

**TABLE 52-3. METHODOLOGIC GUIDELINES FOR CONDUCTING AND REPORTING COST ANALYSES**

Several issues merit further discussion. Specifically, the setting of the analysis is particularly germane for economic analysis conducted “alongside” clinical trials. A number of factors may affect the analysis, including perspective of the investigators (directly or indirectly related to study sponsorship), variations in experience with the drug, hospital policies, practice patterns, and reimbursement. [13,24,27] In addition, patients treated on clinical trials are treated under “ideal” circumstances, which may not reflect broader practice. Patients are also carefully selected and may not be representative of the larger community.

The learning curve with new technologies or drugs may be quite steep, resulting in an exaggeration of cost early on. [27] However, full accounting of start-up costs is not routinely examined. In a recent review of 181 articles of cost analysis, only 14 (14.4%) studies including actual cost data (97 of 181) accounted for start-up costs. This coupled with provision of general overhead costs (administration and occupancy) can account for more than 50% of all expenditures. [28] Results also need to be reviewed within the context of current practice. Changes in site or type of care may vary considerably with time. The reader needs to consider whether the alternative strategy is “still” cost effective.

Despite efforts to standardize the methodology as well as the interpretation of results, the potential still exists for misinterpretation or misuse of results. The concern is that these types of analyses will control resource allocation rather than inform or shape policy. [14] Another concern is that when faced with budgetary constraints, CEA will result in inequity. Ubel and coworkers [29] conducted a survey of prospective jurists, medical ethicists, and experts in medical decision making, in which participants were asked to select between two screening procedures. The first was less effective, but with the hypothesized budget, could be applied to everyone. The second was twice as expensive, but more effective, resulting in more saved lives. Within the budgetary constraints, however, only half the population could receive the clinically “better” test. More than half of the respondents selected the less effective test, the majority of which selected on the basis of “fairness”—availability to the entire population versus only a subset of the population (even if randomly defined). The potential for inequity or the appearance of inequity must be addressed in future analyses.[29] Hillner describes the added dilemma for clinicians. CEA operates on the utilitarian principle of maximizing net benefit for all members of the population, which implies that while some may benefit, others may not. In contrast, the clinician's perspective is to maximize the health status of his or her patient—independent of the effect on other patients or resource distribution. [10] As noted, economic evaluation is designed to inform policy, not directly control the patient–physician relationship.

**Applications in Pediatric Oncology—Progress and Challenges**

Most of the clinical economics literature in pediatrics has relied on cost identification (or minimization) techniques. Two examples from large cooperative group analyses illustrate the bridge between economic evaluation and clinical decision making. Green and coworkers, [30] from the National Wilms' Tumor Study Group, compared two treatment regimens for children with newly diagnosed Wilms' tumor that varied by treatment duration (short, 6 months; long, 15 months). Although 4-year relapse-free survival did not significantly differ by treatment duration, the annual total cost (direct medical cost, estimated from relative value units and Medicare charges) for the short duration was 50% of that of the long duration, with an estimated aggregate savings of $730,000 per annum. Although indirect costs were not included, the authors estimated that based on the shorter duration of treatment, these costs would also be substantially lower. [30]

In a second example, Bennett and coworkers[31] recently reported a cost analysis of filgrastim in children with T-cell leukemia and advanced lymphoblastic leukemia. This study was conducted retrospectively, using data on resource use from participants enrolled in a Pediatric Oncology Group randomized clinical trial. This study is exemplary for several reasons. First, costs were estimated based on resource use, tabulated on study case report forms. This resulted in minimal additional work from the cooperative group personnel. Second, cost drivers were identified, a process that could be replicated in prospective analyses. Finally, sensitivity analysis was performed to confirm findings. The authors also carefully acknowledge the limitations of this study—principally the lack of statistical power in the economic analysis due to the extent of variance in cost estimates. Nevertheless, these studies in pediatric oncology and others in adult oncology lend credence to the concept of incorporating economic evaluation within or alongside clinical trials. [32,33] and [34] To minimize burden to study personnel and decrease the potential for missing data, economic studies must be carefully crafted. For example, the identification of cost drivers, responsible for 90% of the costs, eliminates the need to collect information on all costs.[35] Design of all reporting forms to collect this information prospectively also minimizes the demands on local or group-wide staff. The 1998 guidelines developed by the National Cancer Institute–American Society of Clinical Oncology Economics Workshop outline the steps required to conduct cost analyses in the context of clinical trials. [36]

Although many CEA and cost utility analyses have been performed on alternative treatments for adult cancer patients, virtually none has been reported in pediatric oncology. Several methodologic challenges have limited its application in pediatrics. The first deals with the valuation of future benefit and future cost. *Discounting* refers to the adjustment of future costs and benefits to current (present) value. This is based on the notion that we would prefer to reap benefits sooner and costs later.[22] Although this technique makes some sense in the evaluation of adult care, its application in pediatric oncology is more complicated. For example, the eradication of disease in a 6-year-old (the median age of diagnosis of childhood malignancies) results in an average of 66 years of life saved (based on average life expectancy). The assignment of present value to the return of 66 years of life is difficult to fathom. An evaluation of a wide range of discount rates would be imperative, as illustrated by Goodwin and Shepherd [37] in a recent review of the economic impact of lung cancer. Moreover, the downstream “costs” of the disease and its treatment (in human and monetary terms) in pediatric oncology are not yet well described. What proportion of childhood cancer survivors will enjoy full life

expectancy and with what morbidity? This relates not only to cancer-related morbidity but also to other noncancer illnesses for which the survivor would be at risk due to extended survival. The use of a shorter time horizon (e.g., 1-year, 5-year) for which outcomes are better elucidated, although methodologically easier, ignores the long-term survival benefit. Alternative outcomes including healthy-years equivalents or save-young-life equivalents hold particular promise in evaluating outcomes in children.[20] Decision analysis or other simulation techniques, based on probability estimates, also are useful in determining which factors might have the greatest impact on cost,[38] and inform investigators about the most critical elements to be assessed in prospective clinical trials. [39,40 and 41]

There have been several other impediments to conducting economic analyses in pediatric oncology. Due to myriad methodologic challenges, the development of evaluation tools including quality of life tools for use in pediatric populations has lagged behind that in adult oncology. [42] In addition, although the majority of pediatric oncology patients are enrolled on clinical trials, the collection of information on nontherapeutic end points of trials has been disappointing. Potential strategies to overcome this, included limiting data collection to key cost drivers and restricting participation to those institutions with the capacity (personnel) to complete data, have been proposed as strategies to enhance this type of research. An important caveat to the “limited-institution approach” is the representativeness of the findings to institutions of all sizes. In addition, it has been difficult to secure financial support for quality of life or quality-adjusted economic analyses in both adult [13] and pediatric oncology. An alternative source of funding, industry-sponsored research, has until recently not been tapped in pediatric oncology in large part due to limited interest in pediatric-specific research. Recent regulatory changes (i.e., the Food and Drug Administration Modernization Act of 1997 and the 1998 pediatric rule; see http://www.fda.gov/) requiring more pediatric-specific data may yield more opportunities for research as well as increased industry partnering to enhance drug development and testing in pediatric oncology.

Additional challenges in pediatric oncology include the relatively small number of patients and the heterogeneity with respect to diagnosis, stage, and treatment. Adequate information on clinical outcomes, particularly as it relates to follow-up data, often lags behind as treatment innovations continue, rendering some analyses moot or dated. Finally, given the dramatic improvements in disease control, much of the focus in pediatric oncology has been on traditional end points, notably survival. In contrast to adult oncology, most children are treated with curative intent versus symptom reduction or palliation.

### Applications to Resource-Intensive Treatments

Despite the fact that studies have not been performed specifically in a pediatric setting, from a methodologic perspective, the evaluation of resource-intensive treatments, such as stem cell transplantation (SCT), is pertinent to pediatrics.

SCT has been the subject of several studies recently reviewed by Waters and coworkers. [43] In addition to the methodologic considerations outlined above, the analysis of the cost of SCT requires attention to several other issues. These include a delineation of standard treatment (inclusive of supportive care) to allow for an accurate comparison within and across studies. For example, the impact of progenitor source (peripheral blood stem cells, primed or unprimed collections with hematopoietic growth factors, marrow, purged or unpurged) and the cell dose infused was recently shown by Schulman and coworkers [44] to be linked to resource use. Studies also should address the “learning curve phenomenon.” [27] This phenomenon suggests that costs might decrease over time and outcomes may improve with increasing experience with the technology. These two issues are interrelated with the third issue—important interinstitutional differences in comparing the cost of treatments across sites. In particular, the organization and site of care delivery may vary tremendously across sites as well as over time. It is important, therefore, in any economic evaluation to present all of these dimensions of care in a transparent fashion as well as to determine if differences are too great within or across institutions to pool results or compare them. Attempts to control costs in SCT and other applications are discussed in the section that follows.

## INSTITUTIONAL PERSPECTIVE

> “Ever try going through the supermarket checkout line with a $50 bag of groceries and putting $10 on the register and walking out the door? That's what goes on in health care every day.”[45]

By 1990, hospital-based costs accounted for two-thirds of the direct costs of cancer care, substantially more than the proportion of direct costs allocated to hospital care for all diseases (49%).[3] Given increased levels of hospital-based care [and the attendant fiscal implications—particularly since the advent of prospective payment system (diagnostic-related group)] in the early 1980s, there was a growing interest in alternative sites of care, particularly the outpatient setting. In response to diagnostic-related group–based payment, several attempts were made to unbundle care to decrease inpatient length of stay and overall cost and maximize payment. For example, outpatient preoperative evaluations and increased use of outpatient diagnostic testing became commonplace.

This shift to the outpatient sector was financially attractive for many institutions and for cancer programs in particular. Results of a 1990 hospital survey conducted by the American Hospital Association indicated that 68% of outpatient cancer programs made a profit with outpatient cancer care accounting for 10% to 20% of hospital revenues.[46] More contemporary data on the fiscal health of inpatient versus outpatient oncology programs, however, are not available. There is a suggestion that the success of this strategy has been variable by hospital size and type as well as percentage of Medicare patients. Its success also is influenced by accounting assumptions, historical and current, in allocating cost between inpatient and outpatient services. [7,8]

One of the biggest fiscal challenges in moving care from the inpatient to the outpatient sector is the need to reengineer the relationship between cost (total cost and allocation) and payment. As more and more cuts are being proposed in the inpatient arena, administrators and program directors must be mindful of the “stepdown” effect required to make such cuts fiscally meaningful. Specifically, decreases in use must be substantial to offset the marginal cost of empty beds or idle machines. The allocation of overhead to the remaining services must also be handled in a way that does not result in increased cost for the remaining services.

The structure of health care financing was historically built on an inpatient model with the Medicare system being the classic example. Specifically, statutory provisions have prohibited Medicare coverage of most prescription drugs administered in the home and outpatient setting. In a newly proposed benefits structure, Medicare would cover ambulatory i.v. antibiotics, allowing stable patients to be treated in the outpatient setting—providing they do not need home health care, which would *not* be covered. Patients requiring advanced care would be transferred from the inpatient acute care setting to a skilled nursing facility. Many critics of the new benefit argue that it could result in more i.v. antibiotic use because oral drugs are not reimbursed. Financially, this effect is referred to as a *woodwork effect*—“coming out of the woodwork.” Additional impediments include increased copayments and cumbersome administration (if more than once-daily administration), which would limit the access of the benefit to approximately 10% of covered patients. Despite the problems, program proponents argue that the program would ultimately cover itself.[47]

The impact of Medicare reimbursement on cancer care has been the topic of recent controversy. Medicare does not cover oral medications (chemotherapy or supportive care), reimbursing for oral drugs “only when they have the same active ingredients as a non–self-administered anticancer chemotherapeutic drug or biological that is covered when furnished incident to a physician's services.” Virtually all of the stakeholders—physician, pharmacy, patients—are at risk if these agents are not adequately reimbursed. The problems include lost revenue or increased OOP expenses. Alternatively, a return to inpatient care or use of parenteral forms of these agents has been proposed. In a 1999 study of oncologists, nurses, administrators, patient advocates, and public policy leaders, 83% of respondents were very or somewhat concerned with reimbursement; 98% have used oral serotonin antagonists. Strikingly, 75% would increase use if these products were included in the drug benefit.[48] Stinson and colleagues[49] performed a cost minimization study comparing different salvage treatments for platinum-refractory ovarian cancer—with presumed equal efficacy and toxicity [two oral—only one of which was reimbursable (etoposide) because there was an equivalent i.v. formulation; two i.v.]. Total drug costs ranged from $4,477 for altretamine to $18,635 for topotecan. Because of reimbursement, patient costs were $4,477 for altretamine compared with $37 with topotecan. Reimbursement would favor the more expensive alternative if patient costs were the concern. [49]

Despite the adoption of a prospective payment structure of reimbursement for inpatient care nearly 20 years ago, Medicare reimbursement for outpatient care has only recently shifted to fixed payment. Although the process is still evolving, proposals have included grouping all chemotherapy into four ambulatory payment classifications (APCs), with payments based on 1996 claims data. New drugs for which there is no 1996 information were automatically placed in the lowest cost category. Because payments would be based on the median cost in each of the categories, in any category there would be constant winners and losers with expected wide variance. In addition, the proposed structure would bundle supportive care (including antiemetics and growth factors) into payment for related chemotherapy administration procedure. Patient copayments also are likely to vary considerably within each category. [50,51]

As proposed implementation grows closer, there are dire predictions that if this is not done “right,” care that could be delivered in the outpatient or home setting will revert back to the inpatient setting, simply because it will not be economically feasible to deliver it elsewhere. [52] Specifically, the 1998 proposed (APC) fee structure would result in a substantial reduction in Medicare outpatient department revenues. Recent legislation would address some of these issues, especially hitting oncology.[53] By limiting the proposed cost variance within APC to no more than 2 times, the number of APCs would have to increase. Also, new APCs have been

proposed for supportive and adjunctive therapies. Importantly, a 2- to 3-year interim payment method for cancer treatment and orphan drugs has also been proposed at 95% of the average wholesale price (AWP) inclusive of supportive care. An outlier payment system and slowed implementation to stave off the rate of revenue loss have also been proposed.[54]

Although many of these proposed changes have their greatest impact felt in the care of the elderly, it certainly behooves the pediatrician and the pediatric oncologist, particularly those caring for medically indigent children, to watch the unfolding of the Medicare APC system very carefully. Once adopted, it is highly likely that state Medicaid offices will adopt the Medicare payment structure with private insurers not far behind. Pediatric oncologists also must help educate policy leaders about those aspects of care that may be different than is care to adults (on which the reimbursement strategies are based).

For example, the Department of Health and Human Services has recently proposed that the state Medicaid agencies adopt the results of the Department of Justice's pricing study (less than AWP minus 5%). For example, most of the drugs in the study would be reimbursed at 65% to 99% of the AWP. Reimbursement for selected agents, such as the serotonin antagonists, however, has been set at 28% to 30% of the AWP.[55] From the states' perspective, this change in reimbursement would be a welcomed opportunity to curb rising costs, reflecting increased Medicaid rolls since 1997, and to address rapidly rising costs of drugs. In Massachusetts, for example, state spending on Medicaid has increased 40% since 1993, with the largest jump occurring since 1997.[56] For institutions, however, proposed cuts in reimbursement for prescription drugs comes at a time when Medicaid is already sorely underreimbursing for services such as hospital, nursing homes, and home care. The impact on medically indigent patients may be in shifting sites of care or changes in access.

A related but equally important issue is a shift in the assumption of financial risk from purchasers (employers and payers) to providers (including institutions). Historically, in a fee-for-service system, the institution developed charges that reflected direct and indirect cost (overhead) as well as a “cushion” to cover “nonrevenue” units within the facility or cover “bad debt” (charity care or unpaid claims). Risk was assumed almost totally by the purchasers of care—based on “whatever the market would bear.” Under the Medicare and Medicaid programs, institutions traditionally shared more of the financial risk, as reimbursements were set based on 80% of the “reasonable and customary” fee structure. Risk shifting increases under a capitated system (e.g., prepaid health care), although some plans have offered oncology services as a “carve out,” based on a negotiated discount on charges. The introduction of global pricing, as has been implemented for SCT, shifts most or all of the risk to the institution.[57] From an institutional perspective, the determination of a global rate (base price plus outlier) requires a comprehensive knowledge of true costs, payer mix within the program, and reimbursement trends by payer. This information aids in determining the amount of risk a program or institution can assume.

**Locus of Care**

Rising health care costs and shifts in reimbursement from fee for service to prospective payment (e.g., diagnostic-related groups) have led institutions to search for programmatic changes to decrease cost. The emphasis on cost control, coupled with technological innovations, has led to a growing trend of care oving from the traditionally resource-intensive (and expensive) inpatient setting to the outpatient setting. Recent estimates indicate that 85% to 90% of all cancer care now occurs in the outpatient setting.[58]

For example, over the past two decades, there has been a dramatic shift in the administration of chemotherapy to the outpatient setting. It is now estimated that 90% of all chemotherapy is administered in either physicians' offices or hospital outpatient clinics.[59] Availability of improved antiemetic regimens and the development of more portable infusion pumps have enhanced this shift. Wodinsky and coworkers[59] reported a 22% difference in the (direct medical) cost per dose of chemotherapy for outpatient versus inpatient administration of the same protocol-specified chemotherapy, principally due to reduced fixed costs. In addition to decreased cost, Pasmantier and coworkers[60] demonstrated fewer protocol deviations in the outpatient versus inpatient administration of chemotherapy, a difference largely explained by a dedicated facility and trained staff in the outpatient setting in contrast to chemotherapy administration on multiple inpatient units. A direct comparison of quality of care indicators in dedicated inpatient versus dedicated outpatient units would be of great interest given reported cost differences.

In addition to a *complete* shift to the outpatient setting, several hybrid alternatives have been proposed, including delayed hospital admission and early hospital discharge programs. In addition, several specialty units (e.g., day hospital, specialty emergency department) have been proposed to minimize inpatient utilization.

***Delayed Hospital Admission***

In the delayed hospital admission model, hospital admission is restricted to the subset of patients who cannot be managed with routine or intense outpatient management. Farah and coworkers[61] reported that 13 of the 17 patients (76%) in their study receiving fractionated total body irradiation (TBI) were able to remain outpatients until after the last dose of TBI. Of the remaining four patients, two required increasingly intense outpatient care and two required early admission. The authors reported savings estimates of $3,250 per patient, based on lower total hospital charges and ambulance transport.[61] These findings mirror those reported by Applegate and coworkers.[62] Among 68children receiving outpatient TBI, only 14 patients (20.5%) had any reported significant complications. One child required admission before the completion of TBI; one required i.v. hydration.[62]

Mullen and coworkers[63] have also reported the results of delayed hospitalization in a recent study of children with febrile neutropenia. In their study eligible patients initially received a single dose of parenteral antibiotics and close observation in the outpatient clinic. They were then randomized to receive continued parenteral (ceftazidime) or oral antibiotics (ciprofloxacin) and daily monitoring in the outpatient clinic. Hospital admissions were reserved for those patients who were unable to tolerate outpatient management. Among the 73 episodes of febrile neutropenia in 41 patients, 86% of the episodes was managed as outpatient only. The median charges ranged from $1,544 for the group receiving oral antibiotics (outpatient only) to $4,503 for the subset requiring hospitalization. The intermediate group receiving parenteral antibiotics in the outpatient setting incurred charges of $2,039.[63] In contrast to the Farah et al.[62] study, this study included an estimate of direct nonmedical and indirect costs. Surprisingly, direct nonmedical costs were very low. None of the respondents reported increased childcare costs. Similarly, no one reported increased work hours lost because of clinic visits and caring for and monitoring child at home. Of note, data were not shown to evaluate employment status (unemployed, on leave, consumption of benefits) or comparison with existing arrangements. In addition, the costs of informal caregiving were not imputed, although in the outpatient setting, patients' families delivered most of care. This study highlights several important issues in evaluating cost analyses. First, similar to the Farah et al.[62] study, the Mullen et al.[63] study had strict clinical (i.e., low-risk) and nonclinical inclusion criteria. Among the nonclinical criteria, patients needed to live within 1 hour from the medical center and have a reliable caregiver. No information was provided about the refusal rate or the ineligibility, based on “social criteria,” although the lack of a caregiver has been shown by others to be a barrier to outpatient care.[64,65] Second, the reported episodes represented 25% of febrile neutropenia episodes during the 2-year period. One needs to interpret the findings with caution, including the preference for outpatient care and the minimal shifts in expenses. Clinically, although recent studies suggest that a subset of patients with febrile neutropenia can be managed on oral therapy,[66,67] questions still remain about the feasibility of outpatient management, including its medical, psychological, and social suitability.[68]

***Early Hospital Discharge***

In the early hospital discharge (EHD) model, cost savings are realized by a reduction in total hospital days through either modification in clinical criteria or transition to an alternative site(s) of care. Bash and coworkers[69] evaluated the impact of altering the discharge criteria related to granulocyte count in children with febrile neutropenia. Instead of relying on an absolute neutrophil count of 500 cells per mm $^3$, a rising neutrophil count was found to be clinically acceptable and resulted in shortened hospital stays at a “considerable” cost savings.[69] An important aspect of this study was that antibiotics were discontinued at discharge. It was deemed unnecessary to transition any care to the home or outpatient setting.

EHD has also been deemed feasible in instances in which continued care is required. Peters and coworkers[70] highlighted several important aspects of the EHD program for women undergoing SCT for breast cancer: availability of 7-day-per-week clinic for rapid assessment and intervention; information transfer; dedicated staff knowledgeable about SCT issues and education of families; availability of outpatient facility (hotel); and special arrangements for emergency rehospitalization. Specifically, in this model, inpatient beds are 'held' for potential readmissions.[70] Although this approach is clinically compelling, the marginal costs of “holding beds” are not delineated nor are the start-up costs, allocation of overhead for clinical and hotel facilities, costs associated with staffing, or OOP expenses.

As discussed earlier, Eisenberg and Kitz[12] evaluated the impact of EHD for patients with osteomyelitis. This study is exemplary in the delineation of direct medical and nonmedical costs as well as estimates of indirect cost. They demonstrate, for example, that although there is a $510 (1986 dollars) savings per patient based on a decrease in direct medical costs, the actual costs to families increased by $214 (indirect and direct nonmedical). Moreover, although there was a $1,052 decrease in the costs of inpatient hospital care, the (direct medical) cost of outpatient care increased by $746. Insurance coverage and availability of home care would undoubtedly influence patients' participation in EHD programs despite substantial savings to the institution or to society.[12] These findings highlight two distinct but

important forms of potential cost shifting—shifts within cost centers (inpatient to outpatient) and shifts to the patient. For example, Rizzo and coworkers [71] recently demonstrated a 34% reduction in total charges for outpatient SCT compared with traditional inpatient care. However, the reduction in inpatient charges realized with the move to outpatient-based SCT was offset by increases in outpatient facilities' charges. Of note, based on survey results from survivors at 1 year posttransplant, the outpatient group (n = 11 responders) did not report substantially higher OOP expenses or lost income than that of the inpatient group (n = 40). One important caveat to this study was insurers' agreement a priori to cover outpatient-based expenses, including a small daily living allowance. [71]

Given the economic impetus to consider alternatives to inpatient care, several studies have reported the creation of specialty outpatient units to ensure quality care. Benjamin and colleagues[72] describe the role of the day hospital in the management of painful crises due to sickle cell anemia. As a result of care in this setting, there was a 40% reduction in hospital admissions, decreased length of stay, and decreased use of the emergency department. Interestingly, although the unit did not cover its expenses (unclear whether these were direct costs or total costs), the cost savings to the institution were more than $1.7 million. [72] Girmenia and coworkers[73] describe the role of a specialized emergency unit for patients with hematologic diseases (including malignancies) in the management of patients with acute myeloid leukemia. This unit is designed to provide rapid diagnostic and therapeutic intervention in hematologic emergencies. In addition to same-day care, the unit also contained a ward to accommodate short-term hospitalizations. Patients in the postconsolidation phase of treatment spent an average of 66% of their neutropenic days as outpatients (48% to 77% across three studies), rehospitalization occurred in 54% of cases, and 28% of patients with febrile neutropenia tolerated early discharge. [73] Although the details of the unit's cost structure and staffing are not the focus of the report, this type of unit provides patients and their caregivers with a safe alternative to costly inpatient care. Giles and Vaughan [74] recently described a single staff model for SCT care, highlighting both the clinical and infrastructural coordination between the inpatient and outpatient care arenas. Through this program both delayed hospitalization and early discharge have been achieved, with a substantial decrease in length of stay, a dramatic increase in the unit's capacity, and decreased overall costs. [74] These examples highlight the fact that due to the complexity of care and the potential severity of illness,[50] oncology or SCT patients must be cared for in a secure environment with seamless communication between alternative sites of care and immediate access to different types of care (home to clinic to hospital). [70]

All of these strategies are designed to decrease inpatient utilization and resultant inpatient direct medical costs. Less clear is the extent to which these strategies result in a decrease in total cost while maintaining clinical and nonclinical outcomes. Equally underreported is the impact on total revenue. What are the potential pitfalls in the strategy to shift care from inpatient to outpatient settings? One is the incomplete understanding of the actual cost of delivering intensive outpatient care, including labor costs, infrastructural requirements, and the allocation of overhead, as well as the utilization targets necessary to render it economical. The second issue is the historical underreimbursement for outpatient care compared with inpatient care. In addition to not covering selected treatments in the outpatient setting (e.g., oral medications), reimbursement guidelines for outpatient services also are less generous for professional services. The 1992 introduction of the resource-based relative value scale marked a dramatic change from reimbursement based on reasonable and customary charges.[50] The current methodology does not include allocation of overhead or malpractice insurance expenses. Moreover, these guidelines are based on actual face-to-face time of provider with patient but do not cover the extensive time required to evaluate diagnostic studies or plan therapeutic interventions. Although costs may be less in the outpatient setting, institutions may also face decreased revenue unless careful attention is paid to delineation of cost and the negotiations with payers reflect those costs. Another factor is the impact on nonmedical and indirect costs. Because these costs exist outside of the institution, they are not routinely collected and, consequently, not included in many analyses.

## IMPACT ON PATIENTS AND FAMILIES

### Out-of-Pocket Expenses: Direct Nonmedical Costs of Care

It is difficult to get a precise estimate of the financial burden of cancer on families, particularly reflecting the current changes in the site of health care delivery and altered patterns of reimbursement. In addition to techniques outlined by Eisenberg and Kitz, [12] researchers have developed cost diaries to be completed by patients and their families regarding OOP (direct nonmedical costs) and lost wages. In a 1979 landmark study of the pediatric oncology population by Lansky and coworkers, [75] cost information was collected from 70 families of children with cancer. These data revealed that more than half of the families were paying more than 25% of the family's weekly income for nonmedical expenses (inclusive of loss in pay) associated with the child's illness. [75] Factors influencing cost included level of care, the patient's performance status, distance from the treatment center, and family size. In a follow-up study in 1983, Lansky and coworkers [76] demonstrated that costs varied by treatment phase with the diagnostic (early) and terminal phases of illness being very expensive periods. More recently, Barr and coworkers, [14] using prospective cost diaries, demonstrated that family-borne costs represented at least one-third of the average family's after-tax income during the treatment of three pediatric malignancies—acute lymphoblastic leukemia, neuroblastoma, and Wilms' tumor. These findings are noteworthy in a number of ways. First, the relatively high level of family-borne costs exists despite first-dollar coverage in the Canadian system. Second, the *level* of expenditure among study participants is nearly tenfold than the estimated 2% of after-tax income spent by the general population on health care and approaches what is typically spent on food and transportation or shelter. Equally important is the likelihood that this is an underrepresentation of true cost because it does not include the cost of relapse. [14]

Houts and colleagues,[77] in a study of adults receiving outpatient chemotherapy, reported that 28% of patients were spending more than 25% of their weekly income; 14% were spending more than 50% of weekly income. These data, based on a modification of the Birenbaum cost diary, also revealed the regressive nature of OOP expenses; lowest income groups were absorbing the same financial burden as higher income patients. [77] This pattern was also observed in the 1987 National Medical Expenditure Survey[78,79] and the Rand Health Insurance Experiment.[80]

The results of the National Medical Expenditure Survey indicated that among the nonelderly, OOP expenditures accounted for 21% of total health care expenditures. These OOP expenditures represented a 6.6-fold greater burden (as share of income) for low-income families as compared to higher-income families. [78] Dicker and Sunshine[79] demonstrated sobering similarities between older families and lower-income, younger families in the amount of fiscal burden for health care. Specifically, 27% of older families and 20% of younger, lower-income families had total OOP expenses for health of 10% or more of their income—compared with only 4% for younger, better-off families.[79] In addition, family work-loss days were a predictor of the financial burden index for younger, lower-income families.

Increased OOP expenditures for health care have pernicious effects not only for these families but also for the larger society. First, health care expenditures at this level may leave families without enough resources to provide for other needed services. Second, underuse of health care may result in greater expenditures when care is ultimately sought due to more advanced conditions or comorbidity. Bindman and coworkers [81] demonstrated the impact of access to care on hospitalization rates for chronic conditions. In the Rand Health Insurance Experiment, lower-income people elected to receive fewer preventive services and, among those with hypertension, had worse blood pressure control. [1]

### Lost Income

Although not a true OOP expense, lost income also represents a serious problem for cancer patients or their families. This includes lost income from wages (regular and overtime) as well as consumption of benefits (sick time, vacation, or personal time). In the Barr and coworkers analysis, [14] for example, 39% of families reported losing half a day per week of paid employment for the duration of the child's treatment. Mothers were more likely than fathers to lose time from work, refuse promotions, or agree to relocate. Houts and coworkers[77] estimated that 55% of the nonmedical costs of outpatient chemotherapy were in lost wages. In a study of women receiving active treatment for breast cancer, Moore[82] reported that half of the employed women had a decrease in wages and 26% consumed benefits. Similarly, 25% of spouses consumed benefits or took time off without pay. Overall, 60% of families had a decrease in family income due to the illness and treatment. [82]

The financial burden of copayments and insurance premiums is also felt profoundly by the working poor; similar to OOP expenditures, copayments, too, are regressive. Rasell and coworkers[78] reported that low-income families spent five times as much of their income on premiums as did high-income families. This can be explained by the fact that absolute premium costs are the same regardless of income level. [78] Dicker and Sunshine[79] demonstrated that the inclusion of premium costs had a profound impact on the proportion of U.S. families at given expense-to-income thresholds. For example, 5% of younger, low-income families spent greater than or equal to 20% of their income on OOP expenses without premiums, increasing to 7.6% with the inclusion of premium costs. [79]

Children with cancer may be most similar to other children with special health care needs in terms of their use of health care services and the financial impact of their care on their families. Specifically, children with special needs are twice as likely to be hospitalized, and their hospital stays are four times as long as those of other children. They also have five times as many physician visits and are twice as likely to require special educational services. [83] The longer-term impact of childhood cancer in terms of resource use has not been elucidated.

The cost of cancer care to the family varies tremendously by treatment phase. For example, it has been estimated that medical care at the end of life consumes 10%

to 12% of the total health care budget and 27% of the Medicare budget.[84] Because ofthe amount of money spent during this phase, both the kinds of care and sites of care are under greater scrutiny.[85] Changes must consider the potential shifting of costs onto the family.[86] In a studyof children with cancer receiving terminal care at home, Birenbaum and Clarke-Steffen[87] demonstrated that although overall costs are lower when the child is cared for at home, direct nonmedical and indirect costs are higher. Moreover, this study estimated that 12% to 24% of the costs of care were borne by the family.[87] The authors caution that the ability to pay the additional costs may influence the site of terminal care. Most of the existing cost analyses focus on patients in the active phases of treatment. Less clear are the economic consequences of care over time—specifically, the services being delivered and in which settings over the disease trajectory and the duration of survival. The relationship between clinical and nonclinical end points, such as progression-free survival, reduction of symptoms, functional status, and satisfaction with the fiscal realities, warrants further elucidation.

Although most cost analyses rely on the societal perspective, it is important to note that outpatient services may place greater burden on families, even though from an institutional or societal perspective the costs may be lower.[88] In addition to potential increases in OOP expenditures, because direct costs are lower, the “spend down” to meet Medicaid eligibility takes longer with outpatient bills than with inpatient bills. As noted earlier, the financial consequences of cancer can be daunting. Among the uninsured, the Berkman and Sampson[88] study demonstrated that families spend 30% of their income on health care when one of their children is diagnosed with cancer.

Although many investigators have attempted to capture comprehensive information on cost, including direct nonmedical and indirect costs, several participants reported no expenditures or just reported time away from work, a finding unlikely during active treatment.[14,71,75,77] This represents an important methodologic challenge—how to interpret missing data and the problems with assuming zero expenditures. Possible explanations include the burden to families of recording this type of information when they are maximally preoccupied with the clinical issues. Some OOP expenditures individually may seem trivial—the full impact, however, is in their aggregation and the extent to which they exceed usual household expenditures. Many may not recognize that they have already made accommodations (job change, reduction of hours), highlighting the need to compare their work status and income with the premorbid state. For example, if one of the parents is already not working, there may be no additional childcare expenses because the accommodation has already occurred. In the absence of complete patient reports, imputed cost, based on the methodology outlined by Eisenberg and Kitz[12] may be particularly helpful.

### Informal Caregiving

Although the determination of direct nonmedical expenses and lost wages poses methodologic challenges, it pales in comparison to the estimation of the cost or value of informal caregiving, generally provided by patients' families. This type of care is uncompensated (in monetary terms) and thus lies outside of the market economy.[89] Arno and coworkers[89] estimatedthat the economic value of informal caregiving for adults approaches $200 billion per year. Although the translation of this estimate to pediatrics in general or pediatric oncology in particular is limited at best, it should be noted that parental caregiving for children with chronic diseases is extensive. In the 1997 National Caregiver Survey, 19% of the 817 survey respondents were parents providing care for children, 64% of whom were younger than 20 years. One in three of these caregivers indicated that he or she faced financial needs related to caregiving—this, despite the fact that parental caregivers had higher income levels than other caregivers.[90] Parents caring for children were reported to provide the highest level of care based on hours of care per week and the type of care (related to activities of daily living). In the aggregate, more than 60% of caregivers provided more than 40 hours per week of caregiving. Although 47% of the total sample was employed, 71% of the employed reported working more than 30 hours per week in addition to their caregiving activity.[90]

Caregiving activities vary by the patient's underlying condition[91] and disease stage.[92]The Study to Understand Prognoses and Preferences for Outcomes and Risks of Treatments (SUPPORT) of seriously ill hospitalized adults reported that 31% of families lost most or all of their savings, particularly those with younger, poorer, or more functionally dependent patients.[124]In addition, 29% of the respondents (n = 2,123) lost a major source of income.[93]Similarly, care in the terminal phases of illness has been shown to result in exodus from the labor force as well as more lost time from work among those who remain employed. This appears to be especially true among lower-income families that may not be able to afford alternative care arrangements.[94]

Several studies have begun to evaluate the impact of caregiving on the caregivers, indicating decline in physical health and emotional well-being.[95,96] and[97] The implications of program initiatives shifting care away from institutional-based care to home-based care must include a careful analysis of the true cost—financial and human—of informal caregiving. In addition, the impact of federal cutbacks to paid home health care on informal caregiving must be carefully assessed. The Congressional Budget Office estimates that upwards of $69 billion will have been taken away from home health care in the period from 1998 to 2002.[98] These cutbacks have stemmed from concerns about corruption and excessive payment among home care providers.

### Economic Issues Facing Patients and Survivors

#### *Insurance*

In a recent survey conducted by Cancer Care[99] of 434 cancerpatients/families and 36 social workers, the *primary* concern expressed by both sets of respondents was fear of losing insurance and being underinsured. Among the patients/families, nearly half of the respondents (46%) worried about how to pay medical bills most or almost all of the time.[99] Several recent legislative efforts address insurance issues germane to children with cancer and their families. Under the Consolidated Omnibus Budget and Reconciliation Act of 1987 (COBRA), parents who either lose or leave their job can continue coverage with their group insurance up to 18 months (22 months if disabled). In addition COBRA also allows for continued coverage for up to 36 months for dependents who reach 19 years (or 22 years if they are disabled). Eligible parents are responsible for the payment of insurance premiums. This eligibility applies either until the covered period expires or new coverage is obtained.[100]

The lack of occupational mobility plagues cancer patients and survivors. In a 1995 survey of cancer patients in treatment at M. D. Anderson Cancer Center, 58% of respondents reported that they would not leave their current (employment) position because of the health insurance. The proportion who were “job locked” ranged from 40% employed by small firms to 88% of respondents employed by large firms.[101] The Health Insurance Portability and Accountability Act of 1996 (HIPAA) was designed to decrease the impact of preexisting health conditions on health insurance coverage during changes in employment. The major features of HIPAA include (a) restriction of the waiting period for the previously uninsured to 12 months based on preexisting condition, and (b) elimination of the waiting period for those who had previously been insured (with paid-up premiums). This minimizes the impact of job changes and addresses the problem of job lock. It is important to note that the law does *not* prevent companies from raising the cost of premiums or leaving smaller markets completely.[5] Ongoing evaluation of the impact of this law across employers is imperative.

Additional programs exist for those who are disabled or are uninsured. The Social Security Administration oversees two federally funded programs for the disabled. Although each provides a monthly cash benefit, they are quite different in terms of eligibility. The Supplemental Security Income (SSI) program, originally enacted in 1974, is designed to assist low-income children and adults with disabilities. Applicants must meet criteria for financial eligibility (income and resources) and a disabling condition that results in “marked and severe functional limitations.” The Social Security Disability Insurance (SSDI) program requires a history of employment with payment into the Social Security system through employment taxes as well as a disabling condition. In both cases, prospective beneficiaries must demonstrate that their mental or physical problems prevent them from doing “substantial gainful activity.” The duration of the disabling problem must be at least 12 months or one that may be fatal.

In addition to monthly cash benefits, each program also provides access to health coverage. The mechanism of that coverage may vary from state to state. SSDI recipients automatically become eligible for Medicare after 2 years of SSDI eligibility.

Over the past decade, the number of children on SSI has increased fourfold. Kuhlthau and coworkers recently reported that in a four-state study of Medicaid expenditures, Medicaid paid 2.9 to 9.4 times more for SSI recipients than for non-SSI Medicaid recipients.[125] Moreover, approximately 10% (7.2% to 12.4%) of SSI recipients had high expenditures (more than $10,000 per year), accounting for 63.4% to 81% of Medicaid expenditures. This compares with the non-SSI group in which 0.8% to 1.7% of the population fell within the high expenditure category, accounting for 14.4% to 28.2% of the Medicaid expenditures. More than half of the expenditures were linked to higher use of hospital or long-term care facilities. Children with SSI and high expenditures were more likely to have a chronic medical condition and developmental chronic conditions than those in the non-SSI group. To curb the continued rapid growth of the SSI program, the 1996 Welfare Reform Act (Personal Responsibility and Work Opportunity Act) has included a more restrictive definition of disability, likely to exclude children with less severe disabilities. It is difficult to predict the impact of these changes on childhood cancer patients or survivors. In addition to restricting enrollment, both SSI and SSDI have recently developed work incentive programs to encourage disabled persons to seek employment and ultimately gain financial independence.

### *State Children's Health Insurance Program*

In February 1997, the General Accounting Office reported a 9.6% reduction in the number of children with private insurance in the period from 1989 to 1995, nearly twofold the rate of adults younger than 65 years.[5] The growing number of uninsured children and the steady erosion of free care served as the impetus for the 1997 Children's Health Insurance Provides Security (CHIPS) Act, creating the State Children's Health Insurance Program. Under this budget agreement, Congress allocated $24 billion over 5 years ($40 billion over 10 years) to provide health insurance to low-income children. [102,103] The states were given 3 years to use the first year's installment of $4.2 billion or forfeit their share to those states that exhausted their allotment. The program was flexible in allowing individual states to offer coverage by broadening Medicaid, creating a separate state insurance program, or offering a hybrid of both. This initiative targets families who earn too much to be eligible for Medicaid but too little to afford private health insurance. Recent estimates from the Kaiser Family Foundation suggest that two-thirds of the 11 million uninsured children may be eligible for coverage under these state-run programs. [104] In its implementation,only ten states actually spent their CHIP allotment; 45% of the money remained unspent. Several states reported problems with delayed implementation, inadequate state matching funds to cover the estimated 16% to 35% state share, or inability to identify sufficient numbers of children for the program. [103,105]

Despite these initiatives, however, the number of uninsured children continues to grow. Recent data suggest that 20% of all children do not have health insurance. [106] This increase is explained in part by a continued drop in the number of children with private insurance, particularly for those families earning less than 200% of the poverty level. Despite an increase in the number of children being covered with public insurance from 29% to 33% (1996 to 1997 and 1998 to 1999), the proportion of children with private insurance whose families earned less than 200% of the poverty level dropped from 47% to 42% during the same time period. [107] This may reflect the increasing cost of private health insurance premiums for family coverage. In 1999 alone, premiums increased 4.8%, with an even greater increase seen in small firms (up to 6.9%).[104] Over the3-year period from 1996 to 1999, family premiums increased 19% to $145 per month.

The situation is particularly grave for young adults aged 19 to 24 years. This group is the most likely to lack health insurance (31.8%)—twice the rate at which all Americans lack coverage. They also have the lowest rate of private insurance.[102] Loss ofcoverage from parental policies as well as employment status (inclusive of type of business) explains a portion of lack of insurance. Several studies have demonstrated higher rates of unemployment among young survivors. [108,109] These overall trends are particularly problematic for childhood cancer survivors with (or at risk for) chronic health care issues. [110]

Historically, faced with an inability to meet health care costs, the uninsured or underinsured could turn to charity care as a source of health care. The amount spent on uncompensated care more than doubled over the decade from the mid-1980s to the mid-1990s.[111] Charity care is unevenly distributed among different kinds of hospitals, with government, public, and non-profit facilities (including teaching hospitals) carrying the bigger burden. The impact of uncompensated care has varied by region. In the Northeast, for example, private teaching hospitals accounted for half of all beds. [112] For nonprofit facilities in particular, already strapped financially by other health care reforms, however, the ability to continue to provide uncompensated care has been seriously eroded, despite the mission or tax status of the institutions.[111] Moreover, studies have shown that charity (uncompensated) care has decreased, particularly among providers involved in managed care plans or in areas with high managed care penetration.[6] Elimination of these services could leave the medically indigent without access to services. Conversely, given other fiscal changes, the provision of uncompensated care has a dramatic impact on the institutions attempting to deliver it.

### Discrimination

One of the recurring themes in the survivor literature is the stigma cancer survivors face with respect to employment and insurance issues. This is reflected in the results of a recent survey of 662 adults without cancer in which 18% of respondents indicated that if they were diagnosed with cancer they would not reveal their diagnosis for fear of discrimination.[100] n addition to difficulties obtaining and maintaining insurance coverage, cancer survivors are less likely than are gender-match siblings to be accepted into the armed forces.[113,114] Younger survivors also report differential levels of educational attainment and employment opportunities than controls.[115] Because younger survivors were not in the work force before their diagnosis, they may require specially tailored programs such as educational and vocational training in contrast to rehabilitation and retraining for older survivors. In-depth research is needed to characterize more fully the barriers to employment for childhood cancer survivors.

Under the Americans with Disabilities Act of 1990, people with cancer are protected against selected types of job discrimination, provided that the “essential functions” of the job can be performed. Employees requiring additional time to complete the job functions are also allowed “reasonable accommodation” under the law. In addition, employers cannot ask prospective applicants about their cancer history. [100] This law also prevents discrimination with respect to offered employee benefits. Parents of children with cancer are also protected under this aspect of the law. Of note, however, is that employers are not required under the Americans with Disabilities Act of 1990 to offer health insurance to their employees; if they do so, it must be done fairly. Despite these safeguards, cancer patients and survivors continue to face cancer-based discrimination. In 1995, it was estimated that 2.4% of all federal discrimination complaints were for cancer-related discrimination. [101] More contemporary and more complete information is needed to evaluate the impact of recent legislation, entitlement programs, and vocational programs on equalizing opportunities for cancer patients and survivors. [116] This information is crucial, particularly within the context of the growing numbers of young adults who are uninsured.

## FUTURE CONSIDERATIONS

The current state of the health care system poses many challenges to each of the stakeholders. While these issues are not unique to pediatric oncology, they influence myriad aspects of the lives of patients and their families as well as aspects of the institutions providing or financing care. These challenges can be summarized as follows.

### Policy Level Challenges: Federal and State

- The changing demographics principally due to the increasing size of the aging population will have a dramatic impact on the overall health care bill. Recent projections suggest that costs associated with cancer will continue to rise. Moreover, the number of people with chronic disease(s) also is expected to increase, resulting in a 6.4% increase in health care expenditures. [117] The effect of this growth on employers is likely to result in a growing rate of uninsured Americans with projections as high as 65 million after 2005.[117] What is the likely impact on pediatric oncology? The possibilities include a growing number of uninsured children and young adults, increased competition for resources/allocations from state and federal agencies, and increased caregiver burden due to intergenerational caregiving responsibilities (childcare and eldercare).
- Gaps or impediments in state and federal programs designed to aid the uninsured or underinsured need to be eliminated to enhance coverage for patients on active treatment as well as for preventive care, adequate follow-up care (disease surveillance and management of sequelae), and end-of-life care.
- To ensure quality of care, the number of adolescents and young adults enrolled on clinical trials should be expanded. Access to clinical trials must be assured through continuing changes in reimbursement, while at the same time, clinical leaders must mandate fiscally responsible investigation.
- Reimbursement (from public or private payers) must reflect contemporary sites of care and therapeutic modalities (e.g., APC fee structure, impact of Department of Justice pricing study) to minimize the financial burden to patients (OOP expenditures) and the institutions and providers responsible for their care.
- The economic impact of informal caregiving must be addressed through expanded funding, acknowledging the profound gaps between current allocations and estimated economic value.

### Institutional Challenges

- Given rapid changes in the organization and payment of health care, institutions must become well informed about the allocation of cost (including overhead) by site of care as well as evaluate the impact of reimbursement strategies by payer. This information will aid institutions as they negotiate in a managed care environment.
- Reimbursement must be realigned with current sites of care (e.g., outpatient and home-based care), recognizing key components of care in each setting. Cost-driven strategies to date have focussed on reducing direct medical cost, principally hospital-based analyses. These analyses have been performed without apparent linkage to revenue streams or reimbursement strategies. Programmatic initiatives, for example, designed to change (decrease) length of stay or locus of care trigger reimbursement changes. These changes from a profit and loss perspective may be financially deleterious to the institution, to providers, and to families (driving increased uncovered costs or higher OOP expenditures). The consequences of this include a weakening of the institutions as well as increased impediment to deliver or seek health care services. Existing gaps in coverage, especially in the areas of direct nonmedical costs (e.g., OOP expenditures,

premiums, deductibles, and copayments) and indirect costs (lost wages) must be addressed.

### Methodologic Challenges

- Despite limited experience or methodologic challenges in the collection of fiscal data beyond direct medical cost information, rigorous attention must be placed on the inclusion of direct nonmedical and indirect costs. Compared to direct medical costs, patient-borne costs may seem trivial yet in the aggregate may be extremely burdensome. Moreover, these costs may be an impediment to care.[118] This maybe accentuated in vulnerable populations—rural poor (e.g., decreased access and availability of services), medically indigent, and the uninsured working poor. [119,120] and [121] Policy implications include design of outreach programs to enhance care in remote areas[122] and more minority representation in clinical trials, [121] which maybe associated with both enhanced outcomes and lower costs.
- Studies need to be reoriented to address the fiscal impact of start-up costs, the “learning curve” phenomenon, and woodworking effects of new technologies or infrastructural changes to ensure clinically and fiscally sound broader implementation.
- Additional funding is needed to support quality of life and quality-adjusted economic analyses in pediatric oncology across the continuum of cancer care from prevention to long-term follow-up to enhance the methodologic rigor with which technologies, treatment sites, and alternative therapies are evaluated.

## CONCLUSION

Existing and future fiscal challenges necessitate a thorough education of all parties both to enhance their understanding of the economic consequences of decision making and to ensure that they are equipped to participate as informed, responsible members of the institutions and organizations in which they exist. [10,11,123]

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# 53

# PEDIATRIC CANCER: ADVOCACY, INSURANCE, EDUCATION, AND EMPLOYMENT

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## INTRODUCTION

Over the past several decades, the dynamics of advocacy in childhood cancer have shifted. Decision making and responsibility for the care and treatment of children were once almost entirely under the control of pediatric oncology researchers and the medical care team (MCT)—the treating physician(s), nurses, social workers, and child life providers. So too, investigators and the federal agencies funding and overseeing research controlled the priorities in pediatric oncology research and their implementation. Increasingly, however, families and survivors have reshaped their interactions with the health care system into negotiations, in which collaboration and partnership with professionals have become the norm.[1] In recent years, this collaborative approach has begun to affect pediatric oncology research policy and planning deliberations. As partners in pediatric cancer advocacy, health care professionals can engage and support the energy of families and survivors to advance the shared goals of optimizing treatment and quality of life of children with cancer.

This chapter gives an overview of trends in pediatric cancer advocacy and highlights issues important in case advocacy, offering strategies for health care professionals to help families with access to health care and other services for children and families. Also included is a discussion of national issues in which the participation of patient and family advocates is becoming increasingly important—for example, in the design and conduct of research on children, including patient protections in clinical research, and the factors influencing the development and availability of new treatments. The chapter concludes with a discussion of future directions in pediatric cancer advocacy.

## PATIENT ADVOCACY AND THE CANCER SURVIVORS MOVEMENT

Advocacy as used here refers to a spectrum of actions by patients, families, and professionals aimed at improving the outlook for children with cancer and those that care for them. Case or individual advocacy typically refers to families, survivors, or professionals acting as proxy for individual children to press for optimal care and outcomes. Systems advocacy involves families, survivors, or professionals acting individually or through groups to alter health care and other service delivery for children with cancer and their caregivers at a community level or through local public institutions—for example through a clinic, hospital, school, or insurance plan. Systems advocacy also involves efforts to change public policies that affect the pediatric cancer enterprise ( Fig. 53-1).


**FIGURE 53-1.** Pediatric cancer advocacy opportunities.

Originally grounded in the self-help movement of the 1970s, advocacy activities by parents and survivors grew out of the sense that patients have rights to be active participants in their own health care decision making.[2,3] While the health care system focused on treatment of disease, patients organized groups to provide emotional support and information for self-care that could meet their needs beyond what medical care alone could provide.

Patient advocacy took a dramatic turn with the rise of the human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) epidemic in the 1980s. AIDS activists changed the relationship of the lay public with the medical establishment and eventually influenced the course of clinical research and the processes of drug approval. Lay individuals reviewed and commented on the scientific literature, attended scientific meetings, and gained participation in forums responsible for HIV/AIDS research.[4]

Among cancer patients, a similar model of activism was vigorously pursued by breast cancer patients, who engaged in individual, community, and national advocacy in the 1980s and 1990s. As other cancer-specific groups were formed, they published educational and support materials for patients to help both newly diagnosed patients and long-term survivors manage their care. Programs of groups such as the National Breast Cancer Coalition and the National Coalition for Cancer Survivorship helped adult cancer survivors to acquire more assertive case advocacy skills to use in negotiating their care.[5,6] Professional groups, such as Cancer Care, provided survivors with comprehensive social work services as well as brochures and skills-building workshops for patients' self-advocacy.

As survival rates for cancer patients began to rise in the 1980s and 1990s, concepts of cancer patient advocacy became better articulated and more widespread.[7,8] Increasingly, disease-specific cancer advocates pushed the needs of specific types of cancer patients to the top of the national cancer research agenda. Breast cancer advocates began training survivors in the science of clinical research in National Breast Cancer Coalition's Project LEAD to advance patients' participation in decision making about clinical trials. Breast, prostate, and ovarian cancer advocates have also secured substantial increases in federal research funding and have participated at the highest federal levels of decision making in cancer policy.

Similarly, over the past 30 years, families of children with cancer formed many smaller childhood cancer groups, such as those for leukemia, brain tumors, and neuroblastoma, which could meet patients' and families' needs for education and support (see Chapter 57 for a review of these resources). The first and largest of

these parent groups, Candlelighters Childhood Cancer Foundation, founded in the 1970s, published educational and support materials to help families advocate for their children's care. Other parent-founded, cancer-specific groups, such as the Children's Brain Tumor Foundation, also developed excellent materials sometimes written collaboratively with health care professionals. Many such publications offer practical tips and strategies for coping as well as how to locate patient and professional organizations offering assistance. [9,10,11,12,13,14] and [15]

Information seeking and mutual support are typically the first steps in patient advocacy and have become well established in the childhood cancer community at the individual and community levels. Advocacy on national issues in childhood cancer, however, has tended to fall behind that of groups focused on adult cancers. Several factors are likely to be responsible for this lag. The extreme stress that families experience when managing the diagnosis and treatment of childhood cancer absorbs discretionary resources that might otherwise be redirected to improving health care and other policies that affect patients and families. Furthermore, families and patients affected by a diagnosis of childhood cancer do not form a cohesive group as is the case, for example, with reproductive cancers. Because parents' groups are typically formed around specific types of childhood cancer, fundraising and grassroots organizing also tends to be fragmented among a number of smaller constituencies. There is, however, an increasing interest among parents' groups for “the personal to become political,” whereby lessons learned from parents' experiences are evolving into advocacy on larger issues. Their experiences highlight where and how reform is needed to improve the care and outlook for children with cancer.

## TREATMENT NEGOTIATION AND CLINICAL TRIALS

### Maintaining Parental Roles

Pediatric cancer specialists and parents share a contemporary set of values relating to quality care and follow-up of children with cancer: to ensure that treatment decisions are rationally based to maximize the possibility of cure and survival; to minimize the intrusion of the hospital experience on children and families; to ensure adequate follow-up care; and to normalize patient and family life after treatment to the greatest extent possible.

However, this “therapeutic compact of trust” [16] between parents and pediatric oncology specialists creates a special double bind for parents. In consenting to treat their child's disease, parents must subject their child to intrusive, painful, and risky procedures. In doing so, parents fail to protect their child from pain and harm essentially at the hands of strangers. If, however, parents do not allow their children's disease to be treated, this denial fails to protect their child from life-threatening disease. In either case, parents are dependent on medical professionals to carry out what is fundamentally a parental role—saving and protecting the life of one's child.

Treatment decisions at diagnosis and recurrence are the most important contexts in which parents of children with cancer face this dilemma, and it can be the source of much parental anxiety, distress, and tension with other caregivers. The MCT can help parents maintain their role as protectors and enhance families' bond of trust by enabling a greater balance of power in the treatment negotiation.

One way in which parental roles are now being reinforced is through unprecedented access to medical information through the Internet. Families are increasingly becoming medical consumers from the time of a child's diagnosis, comparing specialists, hospitals, treatment options, and clinical trials. Pediatric oncology professionals are only one source of treatment recommendations and options, which now are available from on-line disease-specific chat groups and listservs, and the Web sites of nonprofit organizations, commercial medical information, pharmaceutical companies, hospitals, and the National Cancer Institute (NCI). Although all Internet information sources are not equally reliable, parents' access to medical information provides a much stronger and more independent base from which they can negotiate treatment decisions.

The greater empowerment of families and survivors that the Internet affords can increase both the value and fragility of parents' trust in an MCT. As a general rule, physicians and nurses typically try to answer families' questions and offer guidance under circumstances that are stressful for everyone. As parents become better educated about their children's options at various institutions, an MCT needs to continue to be seen as a supporter of families and children and not as a promoter—of its institutions, clinical trials, or style of care. The ability of an MCT to strengthen families' confidence in their treatment choices remains a continuing challenge as families become more sophisticated advocates in the care of their children.

### Clinical Trials: Participation of Children in Research Studies

#### *Children as Research Subjects*

The relationship of the MCT to families becomes particularly important and sensitive when enrollment in a clinical trial is the preferred treatment option. Because clinical trials are the means by which new therapies are developed, they are also the locus of important issue advocacy in pediatric cancer. Although some types of pediatric cancer are now cured with current therapies, other types continue to have poor outlook. The need for new and more effective therapies remains critical because current therapies may be ineffective in refractory or resistant disease or because they have toxic immediate or long-term effects.

Because most children with cancer are treated in the context of clinical trials, parents need to understand clearly the difference between standard care and clinical trials. Parents most often see clinical trials as offering beneficial therapy for their children (the “therapeutic misconception”), and for many families, it remains difficult to comprehend the distinction between treatment and research. The confusion between therapy and research makes parents vulnerable to pressure from an MCT to enroll children in trials and makes parents' decisions subject to influence of physicians who are also investigators.

Research in children requires special consideration, and involves public discussion of informed consent and assent, the balance of risk-benefit ratios, and the vulnerability of children, especially sick children. These factors have been identified as requiring regulation and clear guidelines, resulting in the development of formal protections for children as research participants. [17] Although the implementation of these guidelines has served to protect children from harm, it has also resulted in the exclusion of children from research—sometimes from early phase studies of innovative therapies that were being evaluated for life-threatening and fatal diseases.

Accordingly, many concerned with treating children believed that these patient protections in pediatric research actually prevented children with certain diseases from benefiting from medical advances found to be effective in treating similar conditions in adults. To address this barrier to the development of new therapies in children, the National Institutes of Health (NIH) developed guidelines for researchers to encourage the inclusion of children in research. As of 1998, investigators funded by the NIH were required to address the inclusion of children in their research proposals or provide substantial reasons why the inclusion of children in the research project would be inappropriate. Similarly, laws such as the Orphan Drug Act and Food and Drug Administration regulations have promoted testing new anticancer drugs in children. [18,19]

While still acknowledging the unique situation of children as research participants, the NCI's clinical trials program now strongly encourages the participation of children in the early evaluation of new anticancer therapies. Experience has shown that early phase data from children are critical because data from early studies in adults do not accurately predict the reaction and tolerance of children to the same agent for both pharmacokinetic (drug disposition) and pharmacodynamic (drug action) reasons. [20,21] Drug disposition may be different between adults and children due to physiologic differences, such as renal function, and body composition. [22] For example, the children enrolled in clinical trials with all- *trans*-retinoic acid were particularly susceptible to central nervous system toxicities despite the fact that pharmacokinetic patterns of the drug disposition were similar in adults and children. [23,24] A second example has been seen in the limitations of adult antitumor data using paclitaxel to predict activity against solid tumors in children. [25]

Phase I studies in children or adults are designed to define a safe dose and schedule and to characterize the nature and frequency of the toxicities that occur with a new agent. Phase I agents are always given to children with therapeutic intent, and, wherever possible, trials are initiated at doses most likely to provide patient benefit and are based on the best data available.

Although formal analyses of efficacy are limited because of sample sizes used in phase I studies, patients are evaluated for tumor response. Indeed, reviews have shown that 5.0% to 7.5% of children who enter phase I trials achieve either a partial or complete response. [26,27] A recent study reviewed a total of 1,606 patients with cancer who were enrolled in 56 single-agent phase I therapy trials published between 1978 and 1996 and found that the overall objective response rate was 7.9%. These investigators also found the suggestion of a trend toward increasing response rates since 1990, which occurred despite the overall improvement in frontline therapy for pediatric cancers. Possible explanations include better choices of drugs for evaluation in pediatrics as well as faster and more effective dose escalation. [28]

These facts may offer families some hope when children have reached the end of standard or proved treatment options. Some families wish to have their children participate in early phase trials to evaluate new agents, even when beneficial therapy is no longer possible, with the hope that the experience will lead to new and better treatments for children in the future. In general, families tend to be strong supporters of clinical research, often raising funds through parents' groups for research. Some are also involved in the research process itself—for example, by reviewing informed consent documents. The complexity of conducting research on children with cancer continues to require the insights and perspectives from informed parent advocates.

### *Consent and Assent for Clinical Trials Participation*

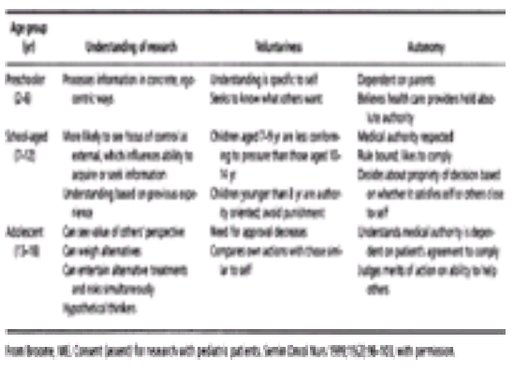
Participation of children as research subjects requires fully informed consent of the parents and, when appropriate, assent by the pediatric participant him/herself. These are critical points of negotiation with an MCT, and parent and survivor advocates can assist the process. For example, advocates can revise consent forms to make them more readable and parent friendly; and they can provide parent-to-parent support during and after treatment decisions. Advocates can assist both investigators and families by helping to maintain the balance between research goals and the protection of patients.

#### Consent

Informed consent is central to the conduct of all clinical trials. Its foundation is set in the Nuremberg Code on the ethical principles of respect for human dignity and individual autonomy.[29,30] The necessary conditions for informed consent include competence, disclosure, understanding, voluntariness, and permission. [31,32] It involves the disclosure of the purpose and nature of the study and the risks and benefits, as well as the treatment alternatives. A genuinely informed consent requires that an individual or his or her (legally authorized) representative is free from coercion or any other form of undue influence that would prevent the consent (or refusal of consent) from being free and voluntary.[33]

In pediatric oncology, the informed consent process for participation in a clinical trial often takes place in an environment of tremendous stress and urgency. Studies are just beginning to provide an understanding of parents' perceptions about the decisions they are making and their ability to make distinctions between medical care and research participation.[34] This research highlights the important need for clinical investigators to clearly explain the research that is being presented. Investigators must communicate a parent's right to choose regarding the child's participation in the clinical trial and must also assess their understanding of the proposed research participation.

In addition to these requirements in pediatric oncology research, the informed consent process has certain unique features because of the differences between children and most adults: different developmental stages that directly affect a child's ability to understand the proposed research; the perceived and real power differences between a child and the health care professionals conducting the research; and the reliance of children on the judgment and beliefs of their parents about medical care and clinical research (Table 53-1).[35]



| Age group (yr) | Understanding of research | Voluntariness | Autonomy |
|---|---|---|---|
| Preschooler (2-6) | Processes information in concrete, egocentric ways | Understanding is specific to self<br>Seeks to know what others want | Dependent on parents<br>Believes health care providers hold absolute authority |
| School-aged (7-12) | More likely to see focus of control as external, which influences ability to acquire or seek information<br>Understanding based on previous experience | Children aged 7-9 yr are less conforming to pressure than those aged 10-14 yr<br>Children younger than 8 yr are authority oriented, avoid punishment | Medical authority respected<br>Rule bound, likes to comply<br>Decides about propriety of decision based on whether it satisfies self or others close to self |
| Adolescent (13-18) | Can see value of others' perspective<br>Can weigh alternatives<br>Can entertain alternative treatments and roles simultaneously<br>Hypothetical thinkers | Need for approval decreases<br>Compares own actions with those similar to self | Understands medical authority is dependent on patient's agreement to comply<br>Judges merits of action on ability to help others |

From Broome ME: Consent (assent) for research with pediatric patients. Semin Oncol Nurs 1999;15(2):96-103, with permission.

**TABLE 53-1. DEVELOPMENTAL DIFFERENCES INFLUENCING A CHILD'S PARTICIPATION IN RESEARCH**

Previously, it was assumed that young people did not have the capacity to give consent to participate in clinical research; parents or guardians went through the informed consent process on their behalf—a process termed proxy consent. Over the last decade, however, medical and legal experts have determined that the participation of children in clinical research warrants special attention—despite the fact that children under the age of 18 are not considered adults and that parents must legally go through the process on their behalf. Now, ethicists, investigational review boards, and clinical researchers accept the joint process of permission of parent or guardian and assent by a child.[36] Supporting this position, the National Commission for Protection of Human Subjects of Biomedical and Behavioral Research established that a child aged 7 years with normal cognitive function is capable of giving meaningful assent and should be involved in some type of assent process.

#### Assent

The Institutional Review Board Guidebook published by the NIH Office for Human Research Protection suggests “that the child should be given an explanation of the proposed research procedures in a language that is appropriate to the child's age, experience, maturity, and condition.” Although the process may vary from institution to institution, this explanation should include a discussion of the purpose of the study, the risks and benefits, and what is expected to happen. In addition to written forms, videotapes, diagrams, and peer discussions should be used. Similar to the informed consent process, assent is an ongoing dialogue between the clinical research team and the child or adolescent, and throughout the clinical trial period the child should have the opportunity to ask questions and obtain clarification when things are not understood. Withdrawal of permission by the child, as well as confidentiality of the research information, should be handled with the same respect and attention as they are with adults.

#### Dissent

In opposition to the assent that is sought for clinical trials participation stands the objection or refusal of a child to participate in research; such opposition is termed dissent. The issue of whether to honor this refusal and consider the dissent binding has not had uniform agreement nationally. Groups such as the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research recommended that researchers honor the dissent of minors. The U.S. Department of Health and Human Services (DHHS) issued a different recommendation, advising that each investigational review board should decide the issue case by case.

In oncology, the dissent of a child participant is not generally considered to be binding when the research, although posing greater than minimal risk, “holds out prospect of direct benefit for the individual subject.” [37] Parents may override the objections of a child, especially if very young. There may be special instances in selected states in which an adolescent is considered emancipated (by marriage or parenthood), however, and this individual may be able to authorize his or her own research participation. In sum, all groups deliberating these questions agree that it is optimal for a child to agree to participate in the research, and furthermore, that the process for accomplishing this task should be carefully planned and implemented, using age-appropriate methods. Given the degree of cooperation that continues to be necessary between researchers, parents, and patients, it is important that MCTs have the cooperation and trust of a child and family in the consent process. It is also important that families and childhood cancer advocates monitor and comment on the evolution of patient protection regulations and their implementation in the care and treatment of children.

#### Further Challenges in Pediatric Oncology Research

Children's participation in clinical research raises additional important questions that are timely for issue advocacy by families. Given recent advances in the molecular understanding of cancer, the opportunities for improved treatment for children are now tremendous. The shared goal of researchers, families, and survivors is to evaluate new agents with the greatest therapeutic promise. Realizing this goal requires the commitment of researchers and attention to numerous complex

ethical, research, and systems challenges. For example, because there are comparatively few pediatric patients eligible for studies, a limited number of new agents can be evaluated at one time. The question of how to prioritize compounds for evaluation that have promising characteristics is a challenge. In addition, traditional study end points may need to be altered to evaluate new classes of agents, such as the angiogenesis inhibitors. New deliberations about quality of life measures as patient-centered endpoints may also be included in the analyses.

Another difficult challenge relates to evaluating new agents on patient populations with high rates of survival on current therapies. Troubling ethical questions arise when considering the safety and efficacy of new agents with unknown side effects if proved treatments result in high cure rates but have problematic long-term and late effects. The advocacy and deliberations of families and survivors are critical to resolving these and other complex issues that arise as new cancer therapies are evaluated.

## CASE ADVOCACY OUTSIDE OF MEDICAL NEGOTIATION: INSURANCE, EDUCATION, AND EMPLOYMENT FOR PARENTS AND SURVIVORS

Once there has been a diagnosis of childhood cancer, families and survivors must also battle myriad insurance, education, and employment issues. For families, problems with reimbursement for treatment, necessary procedures, and services are time-consuming and stressful under already stressful family conditions. For long-term survivors and their families, struggles with access to insurance, needed follow-up procedures and services, and discrimination at school, work, or in the community continue to make life after treatment unnecessarily difficult. In addition to parents and survivors providing case advocacy, parents are advocating to effect system change—for example, by pressing for policies that ensure coverage of the costs associated with participation in clinical trials, described later.

The MCT plays a critical role in assisting families and patients in accessing clinical trials, obtaining appropriate reimbursement, and preparing for life after cancer. With the advent of managed care, nurses' traditional role as information providers has been enhanced to be advocates regarding insurance, school, and employment issues.[38] This section provides the MCT with guidelines to assist families and survivors in overcoming these often demoralizing and frustrating barriers.

Considerable resources are available to help the MCT and families and survivors with insurance, education, and employment issues, including books for cancer survivors.[39],[40] and [41] Disability resources, including a series of factsheets on managed care and rights in the health care system, are available from the Parent Advocacy Coalition for Educational Rights (PACER; http://www.pacer.org/). Pamphlets on job discrimination and the appeal process, although not cancer specific, are available from the Patient Advocate Foundation (http://www.patientadvocate.org/).[42],[43] They also publish a directory of state programs offering financial resources for children, patients, and families.[44]

Brochures and booklets, however, may be insufficient. Barriers that families and survivors of childhood cancer face may require the expertise of professional advocates to evaluate their case, steer them through options, and create blueprints for solutions. For example, the Patient Advocate Foundation has case managers and pro bono attorneys to help resolve insurance, job discrimination, and debt crisis. The Childhood Cancer Ombudsman Program (CCOP) ( gpmonaco@rivnet.net) has specialized in resolving insurance coverage, education, employment, disability rights, and medical options for pediatric cancer patients and survivors for the past 30 years. The Medical Care Ombudsman Program (gpmonaco@rivnet.net) is a volunteer program that handles issues for survivors with medical conditions other than cancer.

CCOP offers the services of pro bono attorneys, a disability and education rights specialist, and the pro bono consulting services of pediatric oncologists, social workers, psychologists, nurses, rehabilitation specialists, and other professionals. CCOP handles referrals from pediatric oncology specialists and family and parents' organizations. CCOP's collaborative approach to the resolution of issues, grounded in case experience and the law, assists patients and families in making informed choices. Their work has benefited the larger pediatric oncology community because parents and professionals involved in these cases go on to learn and disseminate case advocacy skills. The illustrative “cases in point” discussed below are from CCOP case files.

### Insurance: Winning Reimbursement Denials

MCT members serve an important role as advocates in assisting families and survivors directly or by knowing where to refer them for help. An overview of common problems that have legal implications for patients, families, and survivors is provided below along with guidelines on how the multidisciplinary pediatric oncology team can provide assistance by developing strategies to resolve problems.

#### *Patient Care Costs in Clinical Trials*

The standard of care in pediatric oncology is patient participation in high-quality clinical trials. Although the research costs incurred in a clinical trial are covered by a research sponsor (in pediatric oncology, typically the NCI), the routine costs of patient care may not necessarily be covered. Health plans are likely to cover clinical trial participation, however, if it is clear that the trials are cost effective, scientifically valuable, and expected to benefit the patient.

Initially, coverage for patient care costs in clinical trials occurred only in the public sector with interagency agreements for demonstration projects between NCI and the Department of Defense and Department of Veterans Affairs. Later, due to advocates' pressure, private health plans began to formally cover the patient care costs associated with cancer clinical trials. For example, United Health Care entered into an agreement with the Coalition of National Cancer Cooperative Groups to cover costs associated with patients' participation in clinical trials of all diseases. This was followed by the precedent-setting agreement by the New Jersey Association of Health Plans (which includes the state's ten largest insurers) to voluntarily cover participation costs in cancer clinical trials. Although health plans that cover the patient care costs in clinical trials have generally restricted coverage to those approved or funded by the NIH, this trend is changing. Some state legislatures have also mandated coverage for clinical trials sponsored by private insurers, including Rhode Island, Maryland, Georgia, Virginia, Louisiana, Illinois, and (pending) California.

After vigorous advocacy by national cancer patient groups, President Clinton mandated in June 2000, that Medicare would begin coverage for all phases of clinical trials in all diseases, setting a precedent for private insurers. The stumbling block to universal clinical trials coverage has been defining which trials should be covered. Cancer advocates have long pressed for coverage of trials sponsored by both the NCI and the FDA (which includes pharmaceutical trials) and for coverage of all phases of cancer clinical trials to ensure that insurance barriers to full enrollment would not slacken the pace of research on new treatments. Cancer patients' political position has been that denying routine care costs in the context of a clinical trial is inequitable because premiums have been paid and, for many cancers, standard therapies are not curative.

For patients with health plans that continue to deny coverage of clinical trials based on investigational or “experimental” exclusions, guidelines are provided below that explain how the MCT can assist families and survivors in a successful resolution. The MCT typically becomes involved when a family or patient's request for coverage of a medically necessary procedure or medication is denied. Because the MCT is responsible for authorizing medically necessary interventions, parents or patients typically contact the MCT first for advice and assistance on how to resolve the denial. This initial step is often the most important in overcoming insurance-related obstacles.

#### *Letters of Support*

It is usually the responsibility of the MCT to draft a letter of support. Because most pediatric oncology clinical trials build on the foundations of treatments of known benefit established through previous clinical trials, the MCT can demonstrate that patients will be receiving at a minimum the baseline benefits provided by the prior proved treatment. In these cases, the MCT should argue that the treatment is not “experimental” and is investigational in name only.

Health plans flag complex and expensive case categories of treatment, and clinical trials and many pediatric cancer therapies are automatically reviewed. Letters that are general statements of support (for example, those that describe a simple rationale that a certain procedure should be covered for certain reasons) are routinely denied. For standard care interventions, a well-crafted letter of support alone may be sufficient. For more expensive or complicated procedures, however, attaching test results (especially if they determine the appropriateness of an intervention ) and peer-reviewed articles or other documents that provide medical or scientific evidence to support the appropriateness of a treatment for the patient will support the case. A successful letter conforms to the language of a patient's or family's insurance contract. If the contract language is difficult to decipher, an outside agency can provide assistance. Letters should be concise and neutral in tone, focusing on the medical facts of the case and the support for the intervention. [45]

A request for medically necessary treatment both within and outside of a clinical trial can be further strengthened by showing that the reasonable and customary costs and charges for a procedure that an insurance company claims are at variance with the usual charges in that region. Precedent exists for demonstrating that insurers use inappropriate data sets to determine reasonable and customary charges. For example, the American Medical Association and the New York State Medical Society filed a class action law suit in New York State Supreme Court in 2000 against Metropolitan Life Insurance Company and United HealthCare Corporation, claiming that the insurers used data from the Health Insurance Association of American to establish “usual and customary” charges that were lower than the physicians' actual charges in the area. See *American Medical Association v. Metropolitan Life Ins. Co.*, No 00105266 (N.Y. Supr. Ct., filed March 15, 2000). A member of an MCT might request the data set for local zip codes or hospitals on which the alleged baseline charges have been determined and further inquire whether pediatric charges for a procedure were included. In most cases, litigation is unnecessary to obtain reimbursement unless it becomes evident that a health plan's policy is arbitrary in its exclusions of benefits or in its fee schedule.

> **Case in Point:** A health plan refused to pay for participation of a 4-year-old with recurrent Wilms' tumor in a pediatric cooperative group trial. The following points were made to the insurer: (a) the patient responded favorably to standard chemotherapy, allowing tumor excision and completion of chemotherapy and radiation in the protocol; (b) chemosensitivity was established and recurrence was due to the tumor developing resistance to the agents used; (c) a different chemotherapy regimen induced further shrinkage of the tumor, permitting additional tumor excision; (d) recognized standard treatment of Wilms' tumor is to provide high doses of the chemotherapy to which the patient demonstrated sensitivity; (e) although future studies of Wilms' tumor will continue to develop this approach, the therapy has a known benefit, and this patient falls into the category of responders. It was also noted that this therapy cannot be omitted without adversely affecting the child's health outcome. Studies supporting the expected benefit were supplied with the appeal, and coverage was approved.

> **Case in Point:** A long-term survivor of rhabdomyosarcoma required surgical reconstruction of the orbit because of treatment during childhood. The question arose as to whether the procedure was “cosmetic” or “medically necessary” within the confines of the insurance contract. Her physician wrote a letter of support, mentioning the cosmetic benefits of the surgery. Because of this comment, the claims processor linked the surgery to a cosmetic surgery exclusion. The case was resolved by arguing that surgery was medically necessary to correct orbital damage and that the cosmetic benefit was a by-product of the procedure. The patient won the appeal and the reconstructive surgery was authorized—with a delay of 2 months, however.

*Tip:* For a successful letter of support, consider the following suggestions to strengthen arguments for reimbursement for procedures, services, or medication.

- Have the definition and exclusion sections of a patient's health plan readily available; patients and families should provide the plan to the MCT office when they first come for treatment and should be told to give prompt notice of any policy changes or updates.
- Determine whether the policy has an individual managed care option, which permits the patient to obtain treatment outside of coverage limitations if the plan finds it justifiable—for example, when it is less expensive or requires too much bureaucratic effort. An individual managed care option can provide extracontractual benefits without causing a precedent under their policy.
- Use a plan's definition of experimental or investigational; use the data underlying the protocol and supporting publications to demonstrate that the treatment is investigational in name only; emphasize that the protocol advances the established threshold of benefit and that at the very least the patient can be expected to achieve the established benefit; state that the participation of childhood cancer patients in clinical trials is the standard of care.
- Determine whether a plan's exclusions for investigational or experimental interventions are too broad, seemingly to eliminate every pediatric cancer protocol or treatments for patients with a rare late effect; health plans must offer coverage and benefits on a nondiscriminatory basis for all employees.
- If it is not overly burdensome, parents should report back on the scope of their coverage. If some plans cover treatment that others exclude, there may be a pattern of discrimination or unfair dealing. If a company covers a treatment for some forms of cancer and not for others, and if the scientific support for those treatments, which are covered, and those, which are not, is similar, there may be discrimination.
- Provide counter evidence for any treatment guidelines a plan uses that are misguided or out of date.

### *Timeliness of Response to Request for Coverage*

Determine whether a state requires claims to be processed and resolved within a particular time period. Some states require a faster response if the patient is considered or presumed to be “terminal.” If a health plan has not processed a claim or responded within the statutory limits, they may be obligated to pay the costs associated with treatment [see *Harrison v. Aetna U.S. Healthcare, Inc.*, No. 2000CV194-69 (GA. Super. Ct., filed February 16, 2000)]. In that proceeding, the American Medical Association and Georgia physicians claimed that Aetna U.S. Healthcare had broken a state law requiring prompt payment of claims.

*Tip:* If a patient's claim is denied, it should always be investigated. Obtain a copy of the denial letter, even if the denial took place over the telephone. Once the grounds of the denial are available, the team can examine whether the health plan properly investigated the claim. Here are some suggestions on how to do this:

- Obtain a copy of the relevant language in the contract or benefits booklet (usually in the definitions or exclusions sections); if the denial did not refer to the relevant sections, ask the health plan to cite those sections of the policy on which the denial was based.
- Determine the rationale for why the contract language excludes the treatment in question, and whether the application of that rationale to the facts of this patient's condition and treatment raise issues or questions; if so, formulate questions for the family or patient to ask the health plan.
- If the denial is based on lack of medical necessity, write to the health plan to request the following:
  1. Whether the claim was reviewed by the appropriate pediatric oncology specialist; if so, request a copy of the review or memo that the expert provided to the plan along with the board certifications and literature relied on; if not, request the credentials and information relied on by the individuals internal to the company who made the determination.
  2. If the denial was based on in-plan review guidelines, request the relevant guidelines.
  3. If the case was referred for external peer review, request the credentials of the external reviewer to ensure their appropriate level of expertise, inquiring, for example, whether the reviewer routinely performs the procedure, is part of the treatment team for that procedure or its follow-up, or evaluates patients for participation in that procedure.
  4. Ask the health plan to provide the criteria applied to select the reviewer(s) for this case file, any credentialing or re-credentialing system used to ascertain the expertise of the reviewers used, including a copy of the credentialing document and any contractual or other agreement or arrangement the health plan has with the reviewer setting forth the terms of consulting and compensation.

*Tip:* All denials should be appealed. Most denials are likely to result in a successful outcome.

- Check the policy for details on the appeals process, including filing deadlines, whether witnesses can be present at the appeal hearing, and whether affidavits from specialists can be submitted.
- Inquire whether a lawyer can accompany the family or patient to the appeal hearing.
- Request the qualifications of the persons sitting on the appeal panel.
- Determine who makes the final reimbursement decision.
- Ask the patient or family to write a letter requesting the reasons for the denial.
- Again, request references to and copies of the provisions in the plan that the health plan relied on in making their decision.
- Request articles, technology assessment statements, or other materials of any description used or referenced.
- Take full advantage of the health plan's internal appeal process.

  Constructing a support package justifying coverage as outlined above provides information necessary for the appeal process. If possible, the family or patient should attend the appeal hearing along with a member of the MCT to provide medical backup. If the family or patient is incapacitated and unable to attend, and if participation by telephone conference call is possible, a supportive team member should participate. In the unlikely situation that the appeal is still denied, external independent appeals are the next step.

### *Take Advantage of Independent External Review Options*

Investigate the external appeals process thoroughly. Thirty states, motivated by public concerns about managed care and mindful of their obligations to provide fair review process as the gatekeeper for care, now have a process that allows health plan members access to an external and independent review of claims denials. In some states, the procedure only applies to denials of investigational or experimental treatment. In others, the external review process pertains only to health maintenance organizations (HMOs) and to denials of care based on medical necessity criteria. The MCT can determine whether their state offers this service by calling the state office of the Commissioner of Insurance. Although some states may charge a small fee to initiate an appeal, most states require no fees; in all cases, the cost of the independent review is picked up by the health plan.

Patients and families win most external appeals. In CCOP's experience, reasons for denial included a patient being ineligible for the protocol; an intervention was provided as standard treatment when its benefit was unknown and the patient should have been in a protocol; or a patient was treated off study and there was no reason to believe that it could have had any benefit for the child.

**Insurance: Access to Health Plans and Specialty Care**

One of the most frustrating issues facing those affected by childhood cancer is finding and accessing health plan coverage and special services needed for survivors. When dealing with a life-threatening disease, families and patients can find this frightening, time consuming, and extremely stressful. Patients and families have considerable legal protections for access to insurance and specialty care, and the cases and tips below illustrate how these can work.

***Access to Health Plans***

**Health Insurance Portability Act and Accountability Act**

The Health Insurance Portability and Accountability Act (HIPAA) (Public Law 104-191, passed in 1996) allows millions of Americans with preexisting conditions to secure comprehensive health insurance coverage. HIPAA also helps people maintain their coverage if they need to change insurance or jobs and makes insurance more accessible for those working in small businesses.

Under HIPAA, a health plan cannot

- Deny enrollment based on health status, medical condition, claims history, medical history, or genetic information.
- Charge higher premiums among workers; it must provide uniform benefits to all workers.
- Deny individual coverage to a person who leaves a group health plan due to loss of employment or because the new employer does not offer insurance coverage.
- Impose waiting periods or preexisting condition exclusions as long as the individual opted for and exhausted Consolidated Omnibus Budget Reconciliation Act (COBRA) continuation coverage, has had at least 18 months of prior health insurance coverage, and has had no gap in insurance coverage of more than 63 days, excluding employer waiting periods; however, the insurer can charge higher premiums under the individual coverage plan.
- Refuse to provide credit for the time an individual was insured against the preexisting waiting period (e.g., if an individual had prior insurance but it was not in effect when he or she switched jobs).
- Refuse to renew group and individual plans.
- Use genetic information as a preexisting condition unless there is a diagnosis of a related condition (an excellent source for information on genetic issues is the Council for Responsible Genetics, 5 Upland Road, Suite #3, Cambridge, MA, 02140, crg@essential.org).

Under HIPAA, a health plan can

- Impose preexisting condition waiting periods on persons applying for individual plan coverage who have a break in their prior coverage for more than 63 days, excluding any employer-imposed waiting period (including Medicaid).
- Impose a preexisting condition waiting period of 12 months (up to 18 months for late enrollees) on persons applying for group insurance who have no prior insurance. Because the preexisting exclusions are limited to conditions diagnosed or treated within the six months prior to enrollment, however, it is rare that this proves an impediment to survivors of childhood cancer.
- Select, on a nondiscriminatory basis, the coverage and benefits they offer (for example, they can exclude coverage for cancer treatment but would have to do so for all employees).
- Raise an employer's premiums.
- Cap lifetime benefits.

Most states are not enforcing HIPAA, and the federal government has yet to allocate resources for them to enforce it. For more information check the Center for Medicare and Medicaid Services Web site at http://www.cmms.gov/.

**Consolidated Omnibus Budget Reconciliation Act**

COBRA mandates that both public and private employers with 20 or more employees on more than 50% of the working days in the previous calendar year must make insurance coverage available for a limited period of time to employees and their dependents. Under COBRA, employees who have been fired or laid off have a right to continue their group health coverage at their own expense and at a rate no higher than 102% of the employer's group insurance premium for 18 months. Beginning in 1989, it provided the same benefits for people with disabilities for up to 29 months to bridge the gap to Medicare. The premium for the disabled from the months 18 to 29 can increase up to 150% of the premium charged. Listed below are some important facts about COBRA:

- An employer must inform employees of their COBRA rights and notify the group health plan of an employee's death, termination, or reduction in hours. Coverage may extend to a spouse after the death of an eligible employee, after divorce or legal separation, and to a dependent under the same conditions or if the child is no longer dependent during the COBRA period.
- The employee or family member must inform the group health plan of a legal separation, divorce, or a child's no longer being dependent. The employee or beneficiary has 60 days from the date he or she would lose coverage to make a decision about the continuation of coverage.
- COBRA coverage unavailable if the terminated employee is already covered by the spouse's plan, unless the spouse's plan contains any exclusions or limitations with respect to any preexisting condition of the terminated beneficiary [see *Geissal v. Moore Medical Corp.*, (118 S. Ct. 1869, 1998)].
- COBRA coverage may be terminated if the employer stops providing employee group health insurance, if the employee obtains coverage under another plan, including Medicare, or does not pay COBRA continuation premiums.

For further information, contact the Pension and Welfare Benefits Administration at the United States Department of Labor at (202) 219–8776.

> **Case in Point:** An acute lymphoblastic leukemia survivor, 9 years off treatment, was employed in a large service industry. He wished to change jobs and was offered a position in a plumber's apprenticeship program in a small company that did not offer insurance. On speaking with the company's employee benefits administrator, the company agreed to treat his leaving as a reduction in workforce, providing a letter to that effect so that he could be covered on COBRA during his apprenticeship. Because his company was willing to continue his insurance through COBRA, once he obtained employment with a large company that provides insurance, he could take full advantage of HIPAA.

***Access to Specialty Care, Including Care for Late Effects***

As children and adolescents grow into adult long-term survivors, they become the bearers of their own medical histories, transferring information to their next-generation caretakers.[46] Survivors need assistance in finding a new medical team or long-term follow-up clinic to monitor late effects and possible recurrence. The MCT should have a transition process in place whereby patients leaving treatment have access to their medical records and to long-term follow-up resources, including information about treatment and prevention protocols. When survivors have late effects, access to specialty care and related services are necessary for well being.

Each health plan is expected to have a process whereby members can access specialty care if such care is not available within a plan's network of providers. Patients and families may not be aware of this option or know how to use it. The MCT determines when a patient needs specialist intervention and can provide a letter of support to obtain that service. The MCT can make a real difference in the timeliness and quality of such services received by their patients [see *McEvoy v. Group Health Co-Op*, 570 NW2d 397 (Wis. 1997)].

Tools are available to ensure that patients have access to specialty care—for example, the U.S. Department of Labor's actions with respect to Employee Retirement Income Security Act (ERISA) plans.[47] The Department of Labor includes a “quality of services/provider” as a relevant factor in the choice of providers offered. [48]

Children covered under Medicaid have a right to reasonable, adequate, and prompt provision of specialty care services. See *Kirk T. v. Housoun*, HLD 27(12) 77-78 (December 1999), Docket No. CIV. A. 99-3253, 1999 WL 820201 (E.D. PA. Sept. 28, 1999); and *Commissioner v. TakeCare Health Plan*, Inc. No. 933-0290, OAH No. N9412060, Decision of Administrative Law Judge Ruth S. Astle, accepted by Commissioner Keith Paul Bishop on October 29, 1996 (Department of Corporations, State of California, San Francisco). Lawsuits have also set precedents, fining HMOs for not referring pediatric cancer patients to appropriate specialists in a timely manner. See *Nealy v. U.S. Healthcare HMO*, NY, 93 N.Y.2d 209, 711 N.E. 2d 621, 689 NYS 2d 406 (NY 1999), failure to expedite member's transfer to specialty center; *Pappas v. Asbel*, 724 A.2d 889 (PA 1998), petition for cert. filed, 67 U.S.LW 3717 (May 13, 1999)(No. 98-1836); and *Mecca v. PacifiCare of California, Inc.*, 87 Cal. Rtr. 2d. 784 (Cal. Ct. App 1999).

> **Case in Point:** A 20-year survivor of acute lymphoblastic leukemia was diagnosed with morbid obesity, osteoarthritis of the knees and hips, and severe gastroesophageal reflux disease refractory to medical management/nocturnal aspiration. Her physician recommended a gastric stapling procedure not covered under her insurance policy. However, the policy included an “individual medical case management” benefit. This provision is usually applied when the excluded services are less costly for a health plan to provide than is the covered treatment. In these cases, a health plan may agree to authorize the uncovered service extracontractually. This benefit provision was eventually unnecessary because her physicians documented that the oncology treatment she received predisposed her to obesity. The insurer covered the cost based on its definition of medically necessary treatment.

This case illustrates how novel strategies can help survivors access specialty care for late effects. It is also an example of the fact that patients have a clear right to obtain medical records unless they are determined to be potentially harmful or if privileged doctor notes are involved. Withholding records can be the basis of a medical malpractice action [see *Mantica v. New York State Department of Health*, 699 NYS2d 1 (N.Y. 1999), describing how patients have a right to obtain their own medical records from state agencies under the Freedom of Information Act; see also *Weg v. DeBuono*, HLD 28(4) 85–86, April 2000, Nos. 83446, 85512, 200 WL 190518 (N.Y. App. Div. Feb 17, 2000), in which a physician is sanctioned for untimely response to requests for medical records].

> **Case in Point:** A 20-year acute lymphoblastic leukemia survivor with low sperm count as result of treatment is a participant in a long-term follow-up clinic. On learning of a percutaneous sperm retrieval program at a specialized medical center, he underwent the procedure. His self-insured employer program denied coverage on the basis that his condition was not a sickness or injury. With CCOP assistance, he argued that the procedure ameliorated a dysfunction caused by his disease and its treatment, similar to cataract surgery and cochlear implants restoring function, which the health plan covered. See *Saks v. Franklin Covey Co*, EEOC Determination Letter 160-99-0215 April 27, 1999. It was also argued that his case violated the Americans with Disabilities Act (ADA) by excluding coverage for infertility treatments, using as precedent a U.S. Equal Employment Opportunity Commission (EEOC) district office, which determined that an employer's self-insured health benefit plan agreed to cover procedure. His wife's pregnancy was successful.

Federal courts, however, have taken contradictory positions about how the ADA applies to insurance policies, especially those issued under an employer's group health plan. If this issue arises for patients or families, the MCT should consult with hospital counsel to determine whether a health, life, or disability insurance company has violated the antidiscrimination provisions of the ADA.

**Medicaid and Other Entitlement Programs**

Childhood cancer patients and their families are often unaware of benefits available to them under Medicaid and other entitlement programs. For more information see Chapter 52. It is worth noting certain unusual situations occurring under these programs so that the MCT can help patients and families determine whether they qualify, and if so, help them access these programs.

**Medicaid Waivers.** Nursing care can be provided in the home or community and not in an institution if states apply for federal permission to amend their Medicaid programs. Applicants must be financially eligible for Medicaid services to apply for a “home and community-based waivers.” The “Katie Beckett” waiver allows severely disabled children on Medicaid to receive their care at home instead of in a hospital or nursing facility. Approximately 22 states offer Katie Beckett waivers.

Because of recent trends in welfare reform, states are experimenting with other types of waivers to enable patients to receive care out of institutional settings. For example, California recently created a Nursing Facility Level of Care waiver to provide home and community-based care to patients in a subacute facility. California also recently amended its Model Nursing Facility waiver to allow disabled adults who are employed under the work incentives provisions of the Social Security Act to marry while retaining their jobs, Medicaid benefits, and personal assistance services.

> *Tip:* To find out which Medicaid waivers a state offers, contact the Medicaid Eligibility Unit in the county's social services agency or the state Protection and Advocacy unit. State Medicaid directors should be strongly encouraged to apply for or amend their Medicaid waivers to provide home and community-based care.

> **Case in Point:** The parent of a child with relapsed rhabdomyosarcoma was covered under an individual insurance policy with an annual cap on the coverage of prescription drugs of $1,000 per year and no catastrophic coverage relief. The $1,000 cap barely covered one of her chemotherapy sessions. The pharmaceutical companies that supply the drug were contacted to see if the family met the poverty guidelines for assistance. Unfortunately, the family did not meet the poverty guidelines and did not qualify for Medicaid. The MCT checked with the state children's medical services program to determine whether it covered children with physical disabilities who have cancer. Although the MCT's efforts were unsuccessful in this situation, the case serves as an illustration of avenues of assistance.

**Personal Assistance Services.** In-home personal assistance services (help with activities of daily living and housekeeping tasks) are available to eligible disabled Social Security beneficiaries through Title XX of the Social Security Act. States pay the cost for the personal care assistant. These services may also be available to nonbeneficiaries who pay a “share of cost” towards the service. In California, for example, parents who need to work or even need to sleep may obtain these services if their child is disabled. If a parent cannot work full time because of the child's care needs and no suitable assistant exists, putting the child at risk of being placed in an institution, the parent may qualify to be paid as the personal care assistant.

It is clear that unnecessary institutionalization of disabled persons is discrimination per se under the ADA [ *Olmstead v. L.C.*, 119 S. Ct 2176 (June 22, 1999)]. The court made it clear that persons with disabilities should be supported in the least restrictive settings possible, and if institutionalized persons are ready to move into a less restrictive environment, the wait listing should only be for a reasonable time. The Health Care Finance Administration has issued a letter to state Medicaid directors that provides guidance on complying with this Supreme Court decision.

> *Tip:* Late effects clinics need to keep up to date regarding the status of the personal assistant services category. Severely disabled adult survivors receiving Social Security or Medicaid can apply for personal assistance services so that they can remain at home. Application is made through the local or county social services agency. Employers are not required to provide personal assistance services, but personal assistants may accompany a disabled individual to work.

**Mental Health Services.** HIPAA requires that mental health benefits be provided on an equal basis with physical health benefits. However, exceptions and limitations abound in the way the law is applied. To control costs, many county mental health agencies severely limit services to those who are suicidal or violent. When this is the case, it will take advocacy on the part of oncology and late effects programs, local parents of children with cancer, and parent groups concerned with mental health to encourage the development and allocation of sufficient resources to serve their survivors' needs.

**Disability Benefits** Supplemental Security Income (SSI) is generally discussed in Chapter 52. Children's SSI is a welfare program restricted to helping severely disabled children, however, and often families and professionals mistakenly believe it can be awarded for having cancer.

> **Case in Point:** A child diagnosed with cancer at age 3 years had his SSI benefits terminated at age 12 years. SSI provides support during active treatment and continues only if the family has medically documented evidence that the survivor has not medically improved since he was in treatment and still has at least one impairment that meets the written list of disabilities maintained by the Social Security Administration. The family was under the misconception that SSI would continue indefinitely rather than terminating at the end of treatment.

Disability benefits under SSI for adult survivors are different. The standard for receiving benefits requires individuals to demonstrate that they cannot perform any work whatsoever, disqualifying survivors who work part time.

**Case in Point:** A long-term survivor of Hodgkin's disease received chemotherapy and radiation as an adolescent with resulting fatigue, interfering with her ability to attend school and work part time. She has been unable to get disability benefits even with the help of an attorney. CCOP helped her explore state rehabilitation agency assessment and training options, including welfare to work programs. By taking advantage of these options, the survivor found a better job with an upgrade in benefits in a corporation that promotes job sharing.

*Tip:* In the case history above, the survivor was able to work only part time. Survivors in this situation can apply to the state rehabilitation agency to receive assessment, training, and counseling to find more suitable appointments. Rehabilitation agencies also pay for state college tuition, so a survivor might get paid to go to school. The Work Incentives Improvement Act of 1999 can assist survivors on Social Security Administration benefits to return to work while maintaining Medicaid or Medicare benefits.

**Case in Point:** A long-term survivor sought an individual insurance plan for herself and her children, disclosing her history of leukemia in the application. The health plan canceled her policy after 5 months. CCOP informed the health plan that their policy of refusing to cover an individual with a history of cancer regardless of how long they are off treatment from leukemia constitutes a violation of the ADA. The health plan reinstated her coverage and indicated that their underwriting supports denial of insurance during the 10 years after treatment. Because the survivor was more than 10 years posttreatment, she met criteria for coverage.

*Tip:* A letter using the insurer's policy terminology can indicate that a patient is no longer at risk for recurrence—that is, that the patient is statistically indistinguishable from the rest of population, based on the insurer's actuarial data. Such data should be requested from the insurer.

**Education Rights: Individuals with Disabilities Education Act and Section 504 for School-Age Children**

This section presents case histories exercising the legal rights generally described in Chapter 50. It is important that the MCT understands that children have educational rights to help them obtain services and prevent problems from persisting. Because resolving educational issues can be protracted and complex, the MCT should consider seeking assistance from an outside advocacy agency.

The MCT can substantiate in writing the reasons for a pediatric cancer patient's need for special educational accommodations. The Individuals with Disabilities Education Act (IDEA) of 1975, as amended, ensures that children with disabilities are entitled to a free and appropriate public education, according to a set of definitions of disabling conditions. Under IDEA, students are to receive a free and timely educational evaluation, resulting in an individual educational plan (IEP). Most states have free materials describing students' rights under IDEA.

All students with disabilities have a right to attend school when they are medically able to do so, regardless of the nature or extent of the health-related services they may require during the school hours, so long as those services are not required to be provided by a physician. See *Cedar Rapids Community School District v. Garret F.* (119 S. Ct. 992 1999), in which the Supreme Court held that schools are required by the IDEA to provide students with nursing or other health-related services when the students (a) require the health-related services during the school day and would be unable to attend school without such services; and (b) the required services can be provided by a school nurse or other trained individual. Garret F. provides students in need of medical services an equal opportunity to attend school alongside their peers. This legal victory provides meaningful access to the public schools for children with disabilities regardless of their health-related service needs.

Children with cancer may qualify as being “other health impaired” according to IDEA categories of disabling conditions. They may also have hearing impairments, learning disabilities, and other moderate or severe long-term effects of disease or treatment. Appropriate accommodations may include home instruction, resting during the school day, special resource room instruction, or health-related accommodations. Children who are off treatment and whose mental or physical condition does not strictly meet IDEA's categories may qualify for reasonable accommodations under Section 504 of the federal Rehabilitation Act. The MCT can request that a school district's ADA or 504 coordinator be present at an IEP meeting so that the child study team can develop a plan if it is determined that the child does not qualify under IDEA.

A child covered by a Medicaid Managed Care HMO cannot have benefits changed or the scope of benefits reduced without a notice of action and without providing an opportunity for the person to appeal while the coverage is intact. For example, if a child has physical and or neurological damage as a result of cancer and has been receiving nurse assistance in school under Medicaid, the state Medicaid agency may not change nursing support guidelines to eliminate this assistance. A family may file for a “fair hearing” with the state Medicaid office to maintain services until a judge issues a decision. A new IEP meeting should also be requested. If Medicaid coverage fails, school districts are required by law to provide nurses for “health” (not for “medical”) services for students with disabilities.

**Employment Rights**

***For Parents: Family and Medical Leave Act***

The Family and Medical Leave Act (FMLA) entitles eligible employees to take up to 12 weeks of unpaid, job-protected leave and continued benefits in a 12-month period for specified family and medical reasons, including childbirth, adoption, or a family medical emergency. Leave time includes intermittent leave. Research conducted on the financial impact of this provision shows that it would cost employers less to grant the unpaid leave than it would to let an employee quit and hire a replacement. There are regulatory initiatives ongoing at the federal level to expand the value of this policy by permitting unemployment benefits to apply when the Act is invoked.

FMLA applies to all public agencies, including state, local, and federal employers. It also applies to private-sector employers who employed 50 or more employees in 20 or more workweeks in the current or preceding calendar year, and who are engaged in commerce or any activity or industry affecting commerce.

To be eligible for FMLA benefits, an employee must meet the following requirements: (a) work for a covered employer; (b) have worked for the employer for a total of 12 months; (c) have worked at least 1,250 hours over the previous 12 months; and (d) work at a location in the United States or in any territory or possession of the United States in which at least 50 employees are employed by the employer within 75 miles.

A covered employer must grant an eligible employee up to a total of 12 workweeks of unpaid leave during any 12-month period for one of the following reasons: (a) birth and care of a newborn child; (b) placement with the employee of a child for adoption or foster care; (c) to care for an immediate family member with a serious health condition; or (d) take medical leave when the employee is unable to work because of a serious health condition.

A covered employer is required to maintain group health insurance coverage for an employee on FMLA leave whenever such insurance was provided before the leave was taken and on the same terms as those available when the employee was working. Employees may have to pay their share of health insurance premiums while on leave. Additionally, an employee must be restored to his or her original job or to an equivalent job with equivalent pay and benefits. In some instances an employer can refuse to reinstate certain highly paid “key” employees after using FMLA leave when health insurance coverage was maintained. To be considered key, the employee must be a salaried eligible employee (see above) who is among the highest paid 10% of employees within 75 miles of the work site.

Some states also have legislation providing assistance to families whose child has special needs. For example, California enacted the Family Rights Act of 1991, which allows employees to take 4 months of leave every 2 years to care for a family member. Some state laws provide a wider range of benefits. For more information contact the local Wage and Hour Division office of the U.S. Department of Labor.

***For Parents, Patients, and Survivors: Americans with Disabilities Act***

The ADA mandates nondiscrimination against people with disabilities, including those with a history of childhood cancer. The ADA requires that persons with disabilities have equal access to benefits, such as health insurance, and be treated with equal opportunity in all stages of employment, from hiring through each promotion. It also mandates that reasonable accommodations be granted to an individual with disabilities unless it will cause undue hardship on the employer. The ADA covers companies with 15 or more employees, bringing most employers within the jurisdiction of the law. It also protects job applicants from disclosing medical information before being given a conditional job offer so that employers cannot use that information as the basis for denying a job. It is important to note that

confidential medical information in an employee's file is still protected even after the employee leaves a job or retires.

Medical examinations and inquiries are considered unlawful unless they are made after a conditional offer of employment and only if such inquiries are made of all applicants for that job. If an employer withdraws the conditional offer after a medical examination, he or she must show that the company's medical standards are job related and consistent with business necessity.

The ADA is a federal civil rights law, not an affirmative action statute—there are no preferences, quotas, or goals for hiring people with disabilities. It prohibits discrimination by requiring that people with disabilities be given the same opportunities as those of people without disabilities and people without the perception of disability due to past medical history. Employees with disabilities do not receive special job protection once they are hired. An employer can still decline to hire an applicant with disabilities, but the decision cannot be due to the applicant having a disability. The ADA applies to any qualified individual who can perform the essential functions of the job, regardless of whether the job applicant needs a reasonable accommodation from the employer. A qualified individual with a disability means that the job applicant or employee has the requisite skills, education, and experience required for the position. Reasonable accommodations include, but are not limited to, flextime, leave time, job restructuring, and the purchase of equipment so that the employee can perform the essential functions of the job. The employer is not required to provide reasonable accommodations when doing so would cause an undue hardship for the employer—for example, causing administrative disruption or great expense.

If another federal or state law grants greater protection to persons with disabilities than would the ADA in particular circumstances, then the former law applies. The ADA provides minimal uniform protection nationwide, but states may provide greater protection if they so choose. For example, California employers of five or more employees may not discriminate against persons with physical disabilities.

Most important for cancer survivors, the ADA protects someone with a record of impairment. Every cancer survivor has a record of substantial impairment, so the ADA protects survivors whether the cancer is cured, controlled, or in remission, and applies for the remainder of the survivor's life. Unlike other civil rights laws, the ADA also protects those who associate with a person with disabilities. Insurance discrimination against dependents is prohibited, but the scope of coverage afforded dependents may be different from that afforded the employee. A covered employer may not refuse to hire an applicant or fire an employee if (a) that person has a dependent with a disability; or (b) that person has a dependent who is either not covered by the employer's current health insurance plan or might cause increased health care costs. The employer is also prohibited from refusing to insure or providing different insurance conditions for a person solely because they have a dependent with a disability.

The U.S. EEOC enforces the ADA's employment provisions. Persons with ADA employment questions can contact the agency at 1-800-669-3362 or local EEOC field offices, at which complaints can be filed. Regional ADA information centers provide technical assistance at 1-800-949-4232.

> **Case in Point:** During his son's treatment, a father was subjected to multiple acts of discrimination, including intimidation, leading to the father's eventual discharge. The father sought COBRA application papers, and the employer intentionally interfered with his access to company benefits. The EEOC Office of Legal Counsel confirmed to the father that COBRA is indeed an employee benefit and that the employee is entitled to equal access to this benefit. Denial of this benefit formed a basis of an ADA complaint, which on filing, resulted in a settlement for the parents.

> *Tip:* During its 2000 to 2001 term, the U.S. Supreme Court will hear arguments to determine whether the ADA is unconstitutional as it applies to state and local governments. If the Supreme Court rules the ADA unconstitutional as it applies to state, county, and city governments, its antidiscrimination provisions will no longer apply to state employment, including state employee insurance programs, state facilities, and state programs and services, such as Medicaid. Pending the Supreme Court's disposition of this matter, some federal courts have ruled the ADA is constitutional as it applies to states; others have not. A cancer survivor or his or her family must check with local legal counsel or providers with hospital counsel to determine how the law is currently interpreted in their area.

***Employment in the Armed Forces, Police, and Fire Departments***

Survivors of childhood cancer who meet the physical requirements of the particular service may be eligible for a medical waiver to serve in the Armed Forces, reserves, and Reserve Officers' Training Corps, and to obtain admission to service academies. The general rule is that a survivor must be completely free of cancer and off therapy for at least 5 years. For survivors of Wilms' tumor and germ cell tumors of the testes, there is only a 2-year waiting period. See U.S. Department of Defense Directive No. 6130, March 31, 1986, “Physical Standards for Enlistment, Appointment, and Induction.” The courts have expanded this protection to the opportunity for reenlistment of a disabled reservist.

Police and fire departments have their own physical admission standards. However, they usually look at an applicant's current physical condition. Departments cannot ask questions about health or request medical histories until a conditional job offer has been made. See the Web site of the U.S. EEOC ( http://www.eeoc.gov/) and their technical assistance documents on preemployment disability-related questions and medical examinations.

**Protection of Medical and Genetic Privacy**

The current intersection of unprecedented scientific opportunity with the explosion of computer technology opens up new and unique approaches to the treatment and prevention of disease. Powerful information systems are being developed that can store, sort, and retrieve health information in ways previously unimaginable. Health care services are being integrated so that information can be shared across health care providers and institutions. Automated technologies allow a longitudinal medical record to be kept across the life span for individuals and accessed as part of a national health care information infrastructure. [49]

There are tremendous advantages to be gained from the systemic collection and use of electronic health data: better systems can assist patients in making informed decisions; clinical care is improved through the use of faster and more accurate diagnostic methods; instantaneous searches can be done on medical conditions; research results can be rapidly disseminated; and epidemiologic and health services research can be improved. [50],[51] and [52] However, as identifiable health information is increasingly available in electronic form for patient care and clinical research, and as genetic information is thought to have impact on both families and communities, concern is growing about the protections needed to insure the confidentiality of this information.

The right to privacy is an inherent freedom in this country, and as George Duncan states, “It is generally accepted in the United States . . . that ethics for dealing with personal records, including health care records, should have as its core respect for the individual. The person is entitled to a degree of autonomy and is expected to extend that shield to others.”[53] However, this right to informational privacy has never been considered absolute; individual rights do not ignore individual responsibility. A balance must be struck between the right to privacy and the obligation to cooperate in the pursuit of communal goals. The national debate about access to identifiable heath information, the right to privacy, and how to achieve a societal balance will continue for some time given the complexity of the issue and the lack of consensus about what should be contained in a national privacy law.

Policy about the protection of personally identifiable information is fragmented and inconsistent. Informational privacy is protected by the Constitution and some federal legislation, but these protections are limited. [54],[55] Currently, the privacy of medical information is protected principally at the state level, although the specifics of this protection vary widely from state to state. Most of the state privacy statutes are restricted to government-held data and may not have penalties in place for unauthorized disclosure. In contrast, some states have enacted “super-confidentiality” statutes for certain diseases or conditions such as HIV, mental illness, or genetic tests. As of mid-2000, 34 states had passed legislation prohibiting health insurance discrimination, 20 states had passed laws prohibiting employment discrimination, and 11 states had passed some type of law limiting the use and disclosure of genetic information or retention of samples. [56]

At the national level, the path toward a comprehensive privacy law is still unresolved. Although HIPAA required Congress to enact comprehensive legislation to protect electronic health data by August 21, 1999, it was unable to do so and the responsibility fell to the DHHS. [57] Recommendations for legislation have been publicly presented by DHHS, and bills continue to be introduced in Congress. [58] Various legislative proposals conflict, reflecting the competing interests of consumer groups, health plans, practitioners, and researchers. Key to any future comprehensive national privacy legislation will be the ability of these interested groups to come together to acknowledge the significance of identifiable information, develop “best practice” safeguards that balance legitimate use with protections, eliminate differing standards of protections for similar data, and institute penalties for intentional, unauthorized use.

One important issue in discussions of medical privacy is how individually identifiable data should be handled across the spectrum of clinical research, including

clinical trials, genetics, epidemiology, population surveillance, and tissue resources. Often during the debate and drafting of proposed privacy legislation at both the state and national level, the effect on research is overlooked. A number of professional societies, such as the American College of Epidemiology and the American College of Occupational and Environmental Medicine, are developing policy and position statements that include recommendations for the responsible use of research data. After wide consultation with the oncology community, including pediatric oncology, NCI developed recommendations to ensure the confidentiality of identifiable data in research settings and ensure that research perspectives are included in upcoming policy discussions.

At this writing, privacy regulations issued by President Clinton at the end of his term are under review by the Bush administration. These regulations apply to health plans, health care clearinghouses, and those health care providers who conduct certain financial and administrative transactions electronically. The regulations cover medical information in any form, whether communicated on paper, electronically, or orally. The federal regulations also allow stricter state laws to apply. As the national debate on medical privacy and confidentiality continues, recommendations and proposals from the NIH will be essential in assuring protection of personally identifiable information in research.

The medical records of children with cancer can be highly sensitive, potentially exposing not just a patient but an entire family to discrimination. Parents and survivors must continue to advocate in the national discussions on medical confidentiality and data security to ensure the right balance between the privacy protection of research participants and research advancements necessary to improve health.

## CONCLUSION: ACTIVISM AND THE FUTURE OF PEDIATRIC CANCER ADVOCACY

Several converging trends are stimulating new activism in the pediatric cancer community. Since the late 1990s, the NIH, responding to pressure from Congress and from the patient community for greater public involvement in decision making and accountability, has incorporated an increasing number of lay individuals in advisory capacities. The NCI and the FDA have liaison activities offices to organize these efforts. The NCI mandate has brought cancer patient advocates into planning committees, policy and advisory groups, study sections, into a Director's Consumer Liaison Group, into disease-specific planning through the Progress Review Groups, and into committees in the adult cooperative groups and cancer centers. Parent advocates have also participated in the pediatric cooperative groups, including new groups such as the Pediatric Brain Tumor Consortium.

Other factors invigorating childhood cancer advocacy efforts include the merger of the pediatric oncology cooperative groups into a single national group. Parents and survivors have formed a patient advocacy committee to work within the Children's Oncology Group, and professionals, working in conjunction with the National Childhood Cancer Foundation, are playing a more active role in national childhood cancer deliberations. A nonprofit agency founded by parents and survivors, The Children's Cause, Inc. (http://www.childrenscause.org/), was established in 1999 with advocacy and education as its primary mission. In addition, a new Alliance for Childhood Cancer was formed in 2001 to create a national coalition to unify the voice of families, survivors, researchers, and other pediatric oncology professionals on childhood cancer policy.

In this new climate, childhood cancer advocates are taking on important national issues in research and health care policy. They collaborate with other cancer patient groups on common concerns—for example, in the national patient advocacy effort that led to President Clinton's memorandum for insurance coverage of the routine patient care costs in cancer clinical trials (see above). Although the memorandum applied to Medicare beneficiaries, it set important precedents for private insurers to cover pediatric patients in a bill of rights. The cancer advocacy community has also come together to advocate for major increases in biomedical research funding and on issues of medical and genetic privacy, as well as around promising but politically sensitive research issues, such as the scientific investigation of the health potential of stem cells.

Case advocacy expertise continues to be required to help children and families in their struggles with insurance and employment. Building on this experience, patients' rights continue to be a shared priority for the cancer patient advocacy community. Access to health insurance and follow-up care, coverage for specialty care services, and the management of long-term survivors are especially important to childhood cancer advocates.

Advocates are likely to play an even greater role in the design and implementation of clinical research, influencing the pace and feasibility of developing and evaluating new therapies. For example, parents and survivors played an important role in the Brain Tumor Progress Review Group, conducted by NCI and the National Institute of Neurological Disorders and Stroke, which reviewed and planned joint research priorities in brain tumors for the coming years. Strengthening this clinical research capacity of childhood cancer advocates are advocacy training workshops, such as those run by The Children's Cause and the Coalition of National Cooperative Groups along with enhanced communication and access to information through the Internet.

In this new era of collaboration among professionals, parents, and survivors, the entire community hopes that novel solutions will be created to solve the complex challenges of improving treatment, caring for survivors, and protecting the interests of patients and families.

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# COMPLEMENTARY AND ALTERNATIVE MEDICAL THERAPIES IN PEDIATRIC ONCOLOGY

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## INTRODUCTION

As more and more Americans turn to complementary and alternative medical (CAM) therapies for themselves and their children, physicians are being asked a variety of questions for which their formal training may not have prepared them:

- Is this herb safe to use while my child is on chemotherapy?
- Do you think acupuncture might help my child's pain?
- I'd like to try massage to help my child relax during her radiation therapy; can you help me find a pediatric massage therapist?
- My Canadian cousin recommended homeopathic remedies to build up my child's immune system. What do you think?

Physicians need not be experts in every form of therapy to be able to help answer questions such as these; nor do we need to discard critical thinking or evidence-based medicine to understand some of the values that drive families' choices. However, we do need to be aware of the trends in the therapies patients are using, know how to talk with families to elicit a complete history of the different therapies they might be using or considering for their child, understand basic information about CAM practices and CAM providers, and know where to turn for additional information. This chapter provides an overview of CAM in pediatric oncology, acknowledging that this is a rapidly changing field. Other chapters in this text cover closely related topics such as nutrition (see Chapter 42), symptom management (see Chapter 43), psychiatric and psychosocial support (see Chapter 46), listening to families (see Chapter 47), and ethical considerations (see Chapter 48).

## DEFINITIONS

*Alternative medicine* typically refers to therapies that are not generally taught at U.S. medical schools, not provided in hospitals, lack evidence of effectiveness, and not reimbursed by third-party payers[1]; however, because of changes in practice and emerging research, this definition is problematic. For example, over half of U.S. medical schools now offer courses on complementary and alternative therapies or holistic medicine.[2] Many therapies formerly considered alternative, such as acupuncture and hypnosis, are now among the therapies offered in pediatric settings, including children's hospitals. Approximately 75% of pediatric pain treatment services in teaching hospitals in North America now provide one or more therapies (such as acupuncture) that were formerly considered alternative. New evidence is emerging on the safety and effectiveness of a wide range of dietary supplements, massage, and other therapies. As a result of both science and public pressure, increasing numbers of third-party payers reimburse families for services once considered alternative.

Others have defined *alternative medicine* in more anthropologic terms. The Cochrane collaboration, for example, defines it as “a broad domain of healing resources that encompasses all health systems, modalities and practices and their accompanying theories and beliefs, other than those intrinsic to the politically dominant health systems of a particular society or culture in a given historical period.”[3] Implicit in this definition is the view that boundaries within CAM practices and between CAM and mainstream medicine are often blurred and in flux.

Other names for alternative medicine include *complementary*, *folk*, *holistic*, and *integrative medicine*. All have slightly different meanings. Complementary therapies are used in conjunction with mainstream medical therapies. Examples that have found their way into conventional medical settings include massage, support groups, guided imagery, biofeedback, and hypnosis.[4] These therapies are not replacements for medical regimens for serious medical problems but are offered to support the patient and family.

Folk medicine refers to therapies that families or group members provide as part of a family or cultural tradition. Examples include chicken soup for upper respiratory infections, “cold” foods for “hot” illnesses, and religious or ritual healing practices such as coining and sand painting.[5,6] Culturally competent practice requires familiarity with a variety of folk beliefs and healing practices.

*Integrative medicine* refers to the practice of integrating CAM and folk medicine into mainstream practice, based on scientific evidence of their safety and effectiveness.[7] Pediatricians who incorporate chamomile, chicken soup, and other home remedies in their advice about treatments for common conditions such as colic and colds may be said to practice integrative medicine.[8,9]

*Holistic medicine* is closely related. It refers to care of the whole patient—body, mind, emotions, spirit, and relationships—in the context of the patient's values, beliefs, culture, and community. Examples include screening for depression and alcohol use in family members,[10] promoting housecleaning to reduce allergic symptoms, and promoting literacy in the pediatric clinic.[11]

Given the substantial differences in the terms and definitions used to describe complementary and alternative medical care, it is somewhat surprising that epidemiologic surveys show similarities in the rate at which such therapies are used by different patient groups.

## EPIDEMIOLOGY

The percentage of American adults using CAM increased from 34% in 1990 to 42% in 1997, and out-of-pocket expenditures increased 45% during this same period.[12,13] and [14] In a review of 21 epidemiologic surveys of adult oncology patients from around the world, Ernst and Cassileth [15] reported that the percentage of patients using CAM ranged from 7% to 64%; the therapies most often used were mind–body therapies (meditation, hypnosis, biofeedback, guided imagery/visualization), herbal remedies, food supplements, special diets, homeopathy, and spiritual healing. More recent surveys tend to report higher prevalence of use.[16]

In 1994, the percentage of general pediatric patients using CAM was approximately 11%[17]; this percentage increased to approximately 20% in 1997.[18] The prevalence is substantially higher for children and families faced with chronic, recurrent, or fatal conditions.[19,20] In families facing these conditions, rates of CAM use range from 30% to more than 70%, depending on age, acculturation, and access to services.[21] Several surveys have specifically addressed the use of CAM in pediatric oncology patients.

In one study, the percentage of oncology patients reporting the use of CAM was 65%. The most commonly used therapies were prayer, exercise, and spiritual healing; fewer than half the patients had discussed their use of CAM with a physician.[22] In an Australian survey of pediatric oncology patients, 46% of families reported using

at least one complementary therapy; most of these families had used several. The most popular were hypnotherapy, imagery, and relaxation, followed by special diets, dietary supplements, spiritual healing, and homeopathy. [23] In Amsterdam, 31% of pediatric oncology patients reported using alternative therapies such as homeopathy and anthroposophical medicine (excluding mind–body therapies such as visualization, imagery, or relaxation); use of alternative therapies was most common in patients who had relapsed.[19]

The largest survey to date was a questionnaire sent to 583 pediatric oncology patients in British Columbia. Of the 366 respondents, 42% reported using one or more CAM therapies such as relaxation, imagery, massage, Therapeutic Touch (TT), herbal products, and vitamins. Most patients reported that their oncologist was unaware of their use of CAM.[24]

In summary, the use of CAM in pediatric oncology patients is widespread, particularly among those children with relapsing disease, includes a wide variety of therapies, and often is occult to the oncologist.

## PATIENTS' AND FAMILIES' REASONS FOR USING COMPLEMENTARY AND ALTERNATIVE MEDICINE

Conventional therapies for children with cancer are arduous and often have debilitating side effects. Treatment complications are accepted by most families because they are optimistic that standard treatments will cure or control the cancer. However, as the treatments begin to affect the child's quality of life, many parents look toward complementary therapies to alleviate distressing symptoms. Even when conventional therapies are curative, some families turn to special diets and supplements to strengthen their child's immune system and prevent recurrences. And if therapies are unsuccessful, parents begin searching for any therapy that might offer reasonable hope of a cure. Despite the concern that families may delay or reject effective medical therapy for their child's cancer, the vast majority of families use CAM therapies in addition to, rather than as a replacement for, conventional care. [25,26,27] and [28]

In a survey by Fernandez and colleagues,[24] 82% of parents said they needed to know they had done everything possible to help their child; 77% used CAM to boost their child's immune system; 40% used CAM with the intention of curing the cancer, and 35% used CAM to slow the progression of the cancer; approximately one-third said they wanted to use a more holistic approach than standard oncology services provided. Parents are particularly concerned with symptom management and relief of suffering at the end of the child's life—a concern that may not be adequately met with most current mainstream care. [29]

We have been impressed with the amount of time and resources parents at our institution invest in learning about and acquiring complementary therapies. When we inquire about the use of CAM, bottles of vitamins, supplements, homeopathic remedies, and teas appear from pocketbooks and backpacks. The Internet, books, family members, and friends offer testimonials of cancer cures with herbal concoctions and special diets. Often, practitioners of several different healing traditions are consulted, and families purchase product-specific videotapes, audiotapes, herbs, remedies, dietary supplements, and recipes.

For the most part, families seek therapies that are consistent with their values, worldview, and culture and seek care from therapists who respect them as individuals who offer them time and attention.[30,31] Families value highly the care they receive from compassionate, comprehensive physicians who provide individualized care, [32] and they seek additional information on healthy lifestyle choices, dietary supplements, and environmental therapies over which they may exert some control. [33] They also seek care from CAM therapists who offer personal attention, hope, time, and therapies consistent with their values. Families who seek out CAM therapies rarely abandon mainstream care, but they may not feel comfortable discussing those therapies if they perceive the physician to be antagonistic or judgmental toward them. However, they value the opinion of fair-minded, knowledgeable physicians about the safety and efficacy of the products in which they are interested.

## TALKING WITH PATIENTS AND FAMILIES

Despite the increased use of CAM therapies by pediatric oncology services, patients are not substantially more likely to discuss these therapies with their oncologist now than they were in 1990. Families do not communicate about their use of CAM therapies for a variety of reasons ( Table 54-1). The two primary reasons are (a) failure to consider “natural” therapies medically relevant or of interest to a physician, and (b) fear of physician disapproval, leading either to censure, argument, abandonment, or embarrassment. Regardless of a family's reasons for reluctance, it is important for physicians to initiate discussions in a collaborative, systematic fashion.

| |
|---|
| Complementary and alternative medicine considered "natural" or not medically relevant, therefore not worth mentioning |
| Perceived lack of physician interest |
| Fear of censure |
| Fear of being dissuaded from use (argument/conflict) |
| Fear of physician abandoning the child or family at time of great need |
| Cultural practice—may be embarrassed |

**TABLE 54-1. PARENTS' REASONS FOR NONDISCLOSURE OF COMPLEMENTARY AND ALTERNATIVE MEDICINE USAGE**

Opening a discussion about therapeutic options is facilitated by a thorough understanding of the families' goals and values. Treatment goals tend to fall in one of five major areas:

1. Curing the cancer
2. Ameliorating symptoms
3. Preventing symptoms or disease
4. Generally enhancing well being and resilience or reducing toxins and stress
5. Promoting family harmony, cultural solidarity, or a sense of peace

The first three goals are familiar to and shared by most physicians. The fourth goal—enhancing well being and resilience—is frequently emphasized by commonsense cultural precepts (e.g., getting a good night's sleep and eating well to build up one's strength) and by many health care providers (e.g., naturopaths, acupuncturists, chiropractors, massage therapists) who promote their services, not on the basis that they are a cure for cancer, but that they help the body's natural healing systems to work more efficiently. Detoxification is a popular underlying theme in many alternative therapies, although it is an outmoded concept in scientific medicine. The fifth goal is difficult to define precisely but is often an implicit part of many spiritual healing approaches; another aspect of this factor is the drive to try anything that sounds promising in order to ease later guilt: “If only we had tried . . . our child would be alive today.”

In addition to ascertaining the goals of treatment, care providers should assess these important questions before offering a medical opinion about a particular therapy: the name of the therapy, who recommended it to the family, the family's current sources of information about the therapy, their baseline opinion about and experience with it, and their interest in learning more or pursuing this therapy while under the primary care of the medical team ( Table 54-2). For example, they may not be as interested in the scientifically proven efficacy of an herbal product as they are in its potential side effects. Exploring the family's sense of expected end points of therapy and the timeline for achieving their goals can aid in developing realistic expectations and contingency plans.

| Questions | Example 1 | Example 2 |
|---|---|---|
| Name of therapy | Essiac tea | Acupuncture |
| Recommended by | Family of another oncology patient and naturopathic physician | Family physician |
| Information sources | Friends, internet, books, health food store clerk, naturopathic physician, other patients | Physician, internet, magazines, family |
| Purpose of therapy | Kill cancer | Reduce nausea |
| Baseline opinion | Curious, but skeptical | Eager to try it, but would like an alternative to needles |
| Experience | None yet | Patient's father had acupuncture treatment for tennis elbow with good relief |
| Desire for therapy | Neutral, but hopeful | Moderate-strong interest |

**TABLE 54-2. BASELINE QUESTIONS TO ADDRESS WITH FAMILIES ABOUT SPECIFIC THERAPIES**

Having a ready supply of patient information materials about the more commonly used therapies and therapists is invaluable in addressing common concerns. The Center for Families at Boston Children's Hospital and the Blum Resource Center at the Dana-Farber Cancer Institute have developed patient information materials regarding acupuncture, chi kung, chiropractic, homeopathy, massage, meditation, music and sound therapy, naturopathy, Reiki, the relaxation response, TT, and yoga; these materials are available on the institutional internal Web pages for easy access by staff and will soon be available on the external Web pages as well. In addition, the Center for Holistic Pediatric Education and Research in conjunction with the Longwood Herbal Task Force has developed patient information sheets and clinician summary information on the most commonly used herbs and dietary supplements (http://www.mcp.edu/herbal/default.htm). The Rosenthal Center for Complementary and Alternative Medicine at Columbia University also has a very useful Web site for oncology patients: http://cpmcnet.columbia.edu/dept/rosenthal/cancer/.

No matter how well prepared, physicians need to anticipate the inevitable fact that patients inquire about unfamiliar therapies and therapists. For these instances, it is helpful to collaborate with hospital and oncology center librarians, pharmacists, and nutritionists to develop a list of reliable references. There may be hidden resources within the institution, such as nurses or pharmacists, who are also homeopathic practitioners or physical therapists who also practice massage or TT. We have compiled a brief list of general books and Web sites devoted to evidence-based information on complementary therapies in oncology ( Table 54-3). Families have far more respect for a physician whose response to a question about an alternative therapy is “I don't know, but I'll do my best to find out to help your child,” than to a physician who ignores, disparages, or dismisses their concerns.

**TABLE 54-3. RESOURCES FOR CLINICIANS AND FAMILIES**

By better understanding the patient and family viewpoints, experiences, and expectations and by anticipating common questions and informational needs about specific treatments, the physician can offer better advice in a focused, efficient manner. Even after taking a complete history about a particular therapeutic option raised by a family, it is wise to step back and ask in a systematic fashion about all the other therapies the family may have considered before rushing in to offer advice (Table 54-4). Frequently we have found that the initial question raised by the family (e.g., Is Essiac tea safe for a child with a brain tumor?) is the family's way of testing the waters of physician communication and empathy before raising questions about more challenging or sensitive issues.

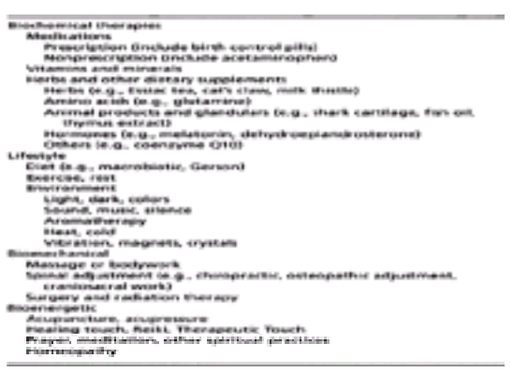

**TABLE 54-4. SYSTEMATIC INTEGRATIVE THERAPEUTIC HISTORY**

## THERAPIES AND THERAPISTS

Just as it is essential when diagnosing a perplexing symptom to have a systematic approach to differential diagnosis (e.g., congenital, autoimmune, toxin, neoplastic, infectious, trauma, psychosocial) and, in taking care of a critically ill patient, to use an organ-system approach to evaluating problems, it is essential when considering therapies to have a systematic approach to considering the potential risks and benefits of different therapies. Although it is tempting to focus solely on the first issue raised by parents, it is prudent to assess thoroughly all the different kinds of therapies the family may be considering before responding fully to the presenting question.

We have found it useful to consider potential therapies in four major categories: (a) biochemical, (b) lifestyle, (c) biomechanical, and (d) bioenergetic. Each of these major categories has several subcategories, some that may be considered mainstream and others that may be considered complementary, depending on cultural circumstances and definitions (Table 54-4). For example, within the general category of biochemical therapies fall medications (both prescription and nonprescription), herbs, vitamins, and other dietary supplements.

### Biochemical Therapy

Herbs, vitamins, minerals and other dietary supplements are increasingly used to treat specific conditions and to promote general health. This is particularly true in pediatric oncology. The questions for which we are most often consulted have to do with the use of herbs and nutritional supplements. Oncologists are well aware of the detrimental effects of combining folate with methotrexate therapy but may be less familiar with the range of other supplements in which parents express interest. One factor complicating responsible care in this area is the rapid shift in popularity—one year it's laetrile, another year it's cat's claw, and within another year, it may

be Japanese medicinal mushrooms—accompanied by intense marketing efforts.

Because of the rapid shifts in the popularity of different dietary supplements, it's important for oncologists to have reliable sources of information to address patient questions in this area. Tables 54-1, 54-2, 54-3, 54-4 and, 54-5 list the sources on which we depend for reliable information. Based on extensive, systematic literature reviews, we have prepared scientific monographs, clinician summary sheets, and patient handouts on some of the most commonly used supplements, and provided linkages to other evidence-based sources of information: http://www.mcp.edu/herbal/default.htm. Hospital pharmacists and nutritionists and regional Poison Control Center toxicologists are also very helpful sources of information.

| | |
|---|---|
| Low fat | Avoids fat, particularly animal fat |
| Block nutrition program | Part of comprehensive approach. Low-fat, high in complex carbohydrates. fruits, vegetables, shiitake mushrooms, kombu, and kelp; occasional fish and free-range chicken; sometimes supplemental vitamins, minerals, and herbs. |
| Gerson diet | Low-sodium, high-potassium foods; frequent raw fruit and vegetable juices; extreme fat restriction; primarily vegetarian diet; coffee enemas (up to five times daily; occasionally includes raw calf liver supplements and other supplements such as potassium, vitamin C, iodine, and thyroid supplements. |
| Livingstone | Primarily vegetarian, whole foods, 50% of which are raw (e.g., up to 1 quart of carrot juice daily); no poultry, smoking, alcohol, coffee, refined sugar, processed foods; selenium extract supplements and a variety of other supplements; bacille Calmette-Guérin vaccine; antibiotics; coffee enema. |
| Macrobiotics | Diet made up of 50-60% whole grains; 20-30% cooked vegetables; 5–10% soups; 5% beans; plus sea vegetables; avoid meat, fish, poultry, and dairy foods; avoid sugar and other sweeteners; avoid tropical fruits, fruit juices, wheat flour, "chemicalized and artificially produced" food and beverages; avoid coffee, alcohol, and ice cream; diet is part of comprehensive, individualized, complete lifestyle management. |
| Vegan | Avoids all animal products, including dairy and eggs. |
| Vegetarian | Avoids meat; includes dairy and eggs. |

**TABLE 54-5. SPECIAL DIETS IN ONCOLOGY**

This chapter does *not* cover supplements commonly used to *prevent* cancer (e.g., selenium, antioxidants, green tea, and garlic).[34] We illustrate here a few of the supplements (a) used to treat cancer itself, (b) used to treat/prevent symptoms and side effects of cancer and cancer therapy, and (c) that may be contraindicated due to interference with other oncologic treatments.[35] Several recent review articles explore these and other dietary supplements in depth.[36,37,38 and 39] The Longwood Herbal Task Force is an excellent Web site with scientific information about herbs and supplements used by oncology patients ( Table 54-3).

***Dietary Supplements Used to Treat Cancer: Essiac, Cat's Claw, Mistletoe, and Shark Cartilage***

One of the most widely used North American herbal cancer remedies is Essiac, which was discovered and promoted by an Ontario nurse, Rene Caisse (“Essiac” reversed). The original herbal formula included four dried herbs: burdock root, the inner bark of the slippery elm tree, sheep sorrel, and turkey (medicinal) rhubarb root. Some newer formulations also contain blessed thistle, cat's claw, red clover, kelp, or watercress.

Despite the numerous case reports exalting the nearly miraculous benefits of Essiac, it has undergone little scientific study. In 1977, the Canadian government sponsored a 5-year clinical trial in the treatment of advanced solid tumors in adults; Essiac was neither curative nor palliative. There are no published prospective controlled trials of the use of Essiac to treat any form of pediatric cancer.

Contamination and allergic reactions are possible. Essiac's reported side effects include diarrhea, nausea, vomiting, headache, and increased urination. It should not be used by pregnant or nursing women, children younger than 2 years, those with a known hypersensitivity to any of its ingredients, or patients with a history of renal stones.

Peruvian rain forest people have used *cat's claw* or *Una de gato* (*Uncaria tomentosa*) for 2,000 years to treat cancer and other conditions. Cat's claw extracts demonstrate antimutagenic, antileukemic, and antitumor effects *in vitro*. Case reports on the effectiveness of cat's claw given to Peruvian children suffering from leukemia have not been followed by published, peer reviewed, controlled trials evaluating the effectiveness of cat's claw either alone or in combination with conventional therapies for leukemia or other forms of cancer.

Traditional herbalists do not recommend cat's claw for children younger than 3 years or persons taking insulin, thymus extracts, vaccines, immune globulin, or sera. An adult who took cat's claw for systemic lupus erythematosus developed renal failure; this may warrant extra vigilance toward renal function in patients who elect to use cat's claw as an adjunctive therapy. A major concern with cat's claw is the potential for misidentification or mislabeling of commercial products with another plant, *Acacia gregii*, which contains a cyanide-based compound. There are also 12 other Peruvian plants called *Una de gato*; throughout the world there are 34 species of *Uncaria* with various medicinal properties, including sedation and hypotension.

*Mistletoe* (*Viscum album* L.) is one of the most widely used herbal cancer treatments in Europe, particularly in Germany and Switzerland, in which it is sold under the brand names, Iscador (Swiss), Eurixor (German), Helixor (German), and Isorel (Austrian). It is typically used to treat adult tumors, such as cancers of the breast, cervix, colon, rectum, and stomach, rather than to treat pediatric leukemia. There are more than 40 studies on the use of mistletoe as a treatment for cancer in adults, primarily for solid tumors. Few of these studies were randomized trials; reports on effectiveness are mixed. No published randomized controlled trials have evaluated the safety and effectiveness of mistletoe in treating children.

In Europe, mistletoe extracts are typically administered via subcutaneous injection or injected directly into solid tumors. Side effects from mistletoe include anaphylaxis, acute fever, and flulike symptoms including nausea and abdominal pain. Oral ingestion by young children may lead to seizures and coma. Due to its tyramine content, mistletoe is contraindicated in patients taking any type of monoamine oxygenase inhibitor.

The most frequent question to the National Institutes of Health (NIH) Center for Complementary and Alternative Medicine when it first opened its doors in the early 1990s concerned the efficacy of *shark cartilage* in curing cancer. In 1994, it was estimated that 50,000 Americans were using shark cartilage, each paying several thousand dollars per year.

Substantial controversy exists about shark cartilage's mechanism of action. Doubts have been cast on its ability to work by oral administration as a natural compound because its large molecular size may preclude intact gastrointestinal absorption. Most studies about shark cartilage have been case reports and case series. A phase I/II trial of the safety and efficacy of shark cartilage in the treatment of advanced cancer in 60 adult patients with stage III or IV previously treated, recurrent, or metastatic cancer (including 16 patients with breast cancer, 16 with colorectal cancer, 14 with lung cancer, eight with prostate cancer, three with non-Hodgkin's lymphoma, one with brain cancer, and two with an unknown primary tumor) concluded that shark cartilage as a single agent showed no anticancer activity. The NIH has recently funded a phase III randomized study of shark cartilage extract in patients with metastatic renal cell carcinoma refractory to immunotherapy. The National Cancer Institute and the M. D. Anderson Cancer Center have undertaken a phase III randomized study of induction chemotherapy and radiotherapy with or without shark cartilage extract in patients with stage IIIA or IIIB unresectable non–small cell lung cancer.

Typical daily adult dosages require dozens to hundreds of capsules daily to reach recommended doses. Shark cartilage is expensive; allergic reactions occur, and significant side effects leading to discontinuation of therapy occur in approximately 10% of those who try it. Safety and toxicity in children have not been evaluated.

We do not typically recommend any of these supplements to pediatric oncology patients. Instead, we provide evidence-based information about the known risks and benefits and also point out the lack of federal regulation ensuring the purity and potency of these products. For copies of our patient handouts on commonly used dietary supplements, please see the Internet site for the Longwood Herbal Task Force, http://www.mcp.edu/herbal/.

***Dietary Supplements Used to Treat and Prevent Symptoms Associated with Cancer and Its Therapies: CoQ10, Ginger, and Milk Thistle***

CoQ10 (also known as *ubiquinone*) naturally occurs in most aerobic organisms; it can also be chemically synthesized. It is a fat-soluble quinone with structural similarities to vitamin K. Since the 1950s, it has been used as an adjunctive therapy for a variety of cardiovascular diseases including congestive heart failure, cardiomyopathy, and doxorubicin-induced cardiotoxicity. Its cardiac benefits are believed to be due to its antioxidant effects and by contributing to membrane fluidity

and stability.

In several studies of rats given anthracyclines (e.g., doxorubicin), pretreatment with CoQ10 was cardioprotective. Treating adults with CoQ10 for 3 to 5 days before chemotherapy with doxorubicin provided protection against decreases in stroke volume, ejection fraction, and cardiac index. Given the consistently beneficial results in animal studies and the generally beneficial effects in adults, CoQ10 may be a useful adjunctive therapy for patients undergoing chemotherapy with cardiotoxic medications, particularly those whose chemotherapy may be limited by the risk of severe cardiac side effects. We often recommend it to patients interested in dietary supplements to reduce the side effects of chemotherapy, but additional studies are clearly needed to determine its optimal role and dosage in pediatric oncology patients.

The most common side effects noted with use of CoQ10 are gastrointestinal, with nausea, anorexia, abdominal pain, and diarrhea reported in fewer than 5% of patients. No neurologic, cardiovascular, renal, hepatic, teratogenic, or mutagenic effects have been noted even in doses as high as 45 mg per kg per day (typical doses for adults are 100 mg daily). There have not been any reports of hypersensitivity to CoQ10. No adverse drug interactions have been reported. However, CoQ10 is a potent antioxidant and there is insufficient research evaluating its potential interactions with chemotherapeutic agents that exert cytotoxicity via membrane peroxidation.

Ginger tea, candied ginger, and dried, powdered ginger are widely used to prevent and treat mild nausea and also as a mild systemic antiinflammatory agent. In animals, ginger effectively enhances intestinal motility and reduces experimentally induced emesis. In rats with chronic, severe inflammatory arthritis, ginger effectively reduced swelling and inflammation. Randomized controlled trials support its use in preventing nausea due to motion sickness, morning sickness, postoperative nausea, and chemotherapy-associated nausea. Its effects appear to be primarily peripheral (on the gut) rather than central (on the central nervous system).

Given its long history of use as a food, ginger is presumed safe. *In vitro*, ginger extracts inhibit thromboxane generation and platelet aggregation in a dose-dependent fashion. Some herbalists suggest caution for patients taking anticoagulants or those scheduled for surgery; however, no clinically significant anticoagulant effects have been documented. Most studies have not shown any impact on platelet aggregation, even with doses as high as 15 to 50 g of fresh, *cooked* ginger, but one study did show reduced clotting in normal volunteers who consumed 5 g of *dried* ginger. No studies have specifically evaluated ginger's safety during pregnancy or lactation or during childhood, but it is on the Generally Recognized as Safe (GRAS) list.

Milk thistle (*Silybinum marianum*) seeds have long been used to prevent and treat hepatobiliary problems and more recently to protect against nephrotoxic drugs. The main active chemical constituent, silymarin, contains silybin (silibinin), silidianin, and silychristin. In animals, silymarin is protective against a number of hepatotoxins—carbon tetrachloride, alcohol, acetaminophen, and viruses. Rats given milk thistle before exposure to cisplatin experienced less nephrotoxicity (as measured by BUN, creatinine clearance, and histology) than did untreated rats. In humans, milk thistle is effective in treating the early stages of alcoholic hepatitis and cirrhosis and as an antidote for Amanita mushroom poisoning. Milk thistle has not been systematically evaluated as an adjunctive therapy for oncology patients with renal or hepatic dysfunction, but many families are interested in using it to protect against the toxic effects of chemotherapy.

Side effects from milk thistle are uncommon. In animals silymarin is nontoxic even at extremely high doses. Rarely, allergic and mild laxative reactions have been reported. Silymarin is also an antioxidant; its potential interactions with peroxidant chemotherapeutic agents and radiation therapy have not been fully investigated.

We often recommend CoQ10 for patients on doxorubicin therapy for up to 6 months after chemotherapy has concluded. We also recommend ginger tea, ginger candy, real ginger ale, or ginger capsules for symptomatic relief from nausea. For patients who are concerned about eliminating “toxic” chemotherapy from their systems, we recommend milk thistle during chemotherapy and for 3 to 6 months after therapy. We also commonly recommend chamomile tea and aloe vera gel as mild antiinflammatory, soothing topical treatment for oral mucositis; chamomile is also commonly used as a natural sedative and gastrointestinal spasmolytic for children undergoing the stress of hospitalization or chemotherapy.

### *Diet Supplements That May Be Contraindicated: Antioxidants and Anticoagulants*

Dietary *antioxidants* are widely promoted to prevent cancer and have been adopted by many patients as a healthy complementary therapy during and after mainstream cancer therapies to enhance resilience and healing. Most studies have shown that antioxidant supplements do not exert the same protective effects as diets rich in fruits and vegetables that contain antioxidants. Also, because radiation therapy and certain chemotherapeutic agents exert cytotoxic activity based on their oxidant effects, antioxidant supplements could theoretically interfere with their effectiveness. [40,41] While studies evaluating potential interactions are under way, we advise families whose children are undergoing radiation therapy or chemotherapy to avoid supplemental beta carotene, vitamin C, vitamin E, lipoic acid, green tea extracts, resveratrol, selenium, pycnogenol (oligomeric proanthocyanidin polymer complexes), and other antioxidants within two weeks of their mainstream therapy.

During phases of treatment in which platelet counts are reduced, dietary supplements that interfere with any aspect of coagulation might be contraindicated. Spontaneous hemorrhages have been reported in adults taking ginkgo, which is a known inhibitor of platelet activating factor. Other herbs that may potentiate platelet aggregation inhibitors include bromelain, Chinese skullcap, garlic, papain, and turmeric. Coumarin is found in several common herbs: horse chestnut bark, sweet clover, sweet vernal grass leaves, and tonka bean seeds. [42] Although fever few has *in vitro* effects on platelet activation, bleeding has not been reported as a complication in any clinical studies.

## Lifestyle Therapy: Diet, Exercise, Environment, and Mind–Body

### *Diet*

Because diet is something over which families have some control, it is one of the lifestyle therapies of greatest interest. Oncologists and nutritionists on the oncology team discuss diet frequently, particularly in terms of caloric needs and the potential for bacterial contamination. Yet families frequently turn to other health professionals, including naturopathic doctors, chiropractors, and acupuncturists about specific diets, and may consult with lay advisers as well.

Several diets for oncology patients have been promulgated to the general public ( Table 54-5). Diets that may help prevent common adult cancers may sometimes be adopted for pediatric oncology patients without sufficient attention to physiologic and developmental needs. [43] Other diets used to promote cardiovascular health, reduce obesity, and avoid other adult health problems may also be adopted by families looking for healthy lifestyle approaches to cancer treatment. These kinds of diets include low-fat, vegan, vegetarian, and high-fiber diets.

Four dietary programs have been widely used to treat adults suffering from cancer: the Block nutritional program, the Gerson diet, the Livingstone diet, and macrobiotics.[44] Both the Block nutrition program and the macrobiotic diet are just one part of comprehensive lifestyle programs for adult cancer patients. The Gerson and Livingstone diets also include coffee enemas and dietary supplements. Gerson includes frequent raw vegetable and fruit juices as well as the juice of fresh calves' liver, and supplemental potassium, iodine, vitamin C, and glandular thyroid. Livingstone, who believed that cancer is caused by a microbe, included whole blood transfusions, gamma globulin transfusions, vaccines, splenic extracts, and antibiotics, as well as numerous vitamin supplements. Nearly all of these diets are low in fat; high in complex carbohydrates, fruits, and vegetables; and devoid of sugar, fats and oils, which may be helpful in many adult conditions but may be inappropriate for children. Severe dietary restrictions run the risk of caloric deprivation and reduced quality of life. For example, the restrictive forms of macrobiotic diets have been associated with cases of scurvy, anemia, and hypoproteinemia. [45]

Although there are numerous testimonials and case reports about the effectiveness of these diets in adult oncology patients, they have not been evaluated in pediatric patients. Michael Lerner's book, *Choices in Healing* (Cambridge, MA: MIT Press, 1994), provides an excellent overview of these special diets and is a good resource for both clinicians and families. We do not typically recommend any of these diets; instead, we refer patients for more intensive consultation with a nutritionist who specializes in pediatric oncology.

### *Exercise*

Although exercise is an important part of a healthy child's life, physical activity in children with cancer may be limited by fatigue, pain, concerns about trauma/bleeding, feelings of self-consciousness, and frustration with diminished strength and endurance. For many children who had been active in individual and team sports, there may also be a sense of loss as their previous capabilities decline and their relationships with teammates are redefined. Several studies have

documented an association between physical activity/exercise and improved psychological outcomes, including reduced measures of depression and anxiety. [46,47,48] and [49] Although intense exercise temporarily lowers natural killer cell activity, regular moderate physical exercise is associated epidemiologically with reduced rates of cancer and enhanced natural killer cytotoxicity [50,51]; the interaction between exercise, immunity, and psychological effects are complex and should be carefully monitored in cancer patients, particularly during periods of immunosuppression. [52,53] and [54] This is a ripe area for additional research.

Recently, there has been a growing interest in Eastern meditative exercises such as yoga, tai chi, and chi kung (qi gong) and their potential therapeutic influences on chronic illnesses such as asthma and cancer.[55,56] Because these exercises can be done slowly and noncompetitively and can be practiced in a group or individually at home, they may be useful for children in various stages of cancer therapy. Theoretically, they have a lower risk of impairing immune function and a lower risk of injuries than do contact sports, running, weight lifting, or other intense exercises or sports. We frequently recommend participation in such exercises to school-aged and adolescent oncology patients.

### *Environment*

Although oncologists pay close attention to the microbial aspects of the environment, particularly for patients undergoing transplants, patients experience a broad range of environmental influences as helpful or stressful. Pediatricians routinely discuss environmental recommendations such as light (phototherapy for jaundice), sound (white noise and vibration for colic, music to reduce stress), and temperature (cold to minimize pruritus).

Boredom and depression commonly follow exposure to isolation and extremely restricted environments. A team meeting in consultation with the family, child life specialists, psychologists, social workers, clergy, and nursing staff may be helpful in anticipating and planning to meet the child's needs for appropriate and helpful stimulation and distraction during a prolonged hospitalization. Developing routines and a predictable schedule during hospitalization may help a child feel a greater sense of control in the face of an overwhelming disease. Medical and nursing routines may need to be modified to meet the child's needs for uninterrupted quiet and rest.

Families may also be interested in a variety of other environmental strategies to help the child feel more comfortable during treatment. Most of these approaches rely on common sense and attention to individual preferences. Bright or dim lights; bright, pastel, or neutral colors; posters, photographs, and other visual art, sounds, music, and television; video games, books, and homework; aromatherapies or avoidance of noxious odors; crystals and magnets; hot and cold packs; and favorite pillows and other objects can all contribute to comfort and decreased anxiety (when they fit with the child's perceived needs) or stress (when there is poor fit with the child's needs).[57,58] Music therapy reduces pain, improves relaxation, and reduces anxiety for hospitalized oncology patients. [59,60,61,62] and [63] These therapies entail few risks and modest costs.

### *Mind–Body*

Mind–body therapies encompass a broad range of practices, including individual psychotherapy, group therapy, support groups, and personal practices such as meditation (see also Chapter 43 and Chapter 46). In the past 20 years, there has been increasing attention to the interaction between psychological states and somatic function.[64] In 1998, Simonton and Sherman[65] reviewed 252 articles addressing behavioral medicine in oncology patients with respect to symptom control, coping and psychosocial adjustment, and immunomodulation. They state that “psychological approaches lead to significant improvements in depression, anxiety, physical functioning and knowledge of the illness.”

Several studies show that relaxation training, biofeedback, mental distraction, hypnosis, and systematic desensitization effectively reduce chemotherapy-associated nausea and emesis.[66,67,68,69] and [70] Psychological factors can also have direct effects on neuroendocrine activity and immune functioning, particularly cellular immunity and NK cell activity.[71,72] The perception of social support also has a clear impact on the adjustment process; several studies show the favorable effects of support groups for patients and families.[73]

Although most of this research has focused on adult cancer patients, there are several strategies that may be beneficial for children with cancer and their families. Hypnosis, guided imagery, progressive relaxation, and biofeedback are discussed in detail in Chapter 43; elements of these techniques can be adapted for children of varying ages and levels of cognitive development. Because parents usually have a profound influence on the coping capabilities of their children, instruction in techniques that they can use to relax, distract, and comfort their children can greatly improve the child's tolerance and cooperation in diagnostic and therapeutic endeavors.[74,75] Furthermore, support groups for parents and siblings of children with cancer may enhance their own psychological well-being and indirectly ameliorate that of the sick child. Small-group instruction in yoga and relaxation techniques for caregivers are now offered through the Dana-Farber Cancer Institute and have received enthusiastic support from families and staff.

## Biomechanical Therapy: Massage and Spinal Adjustments

### *Massage*

There are hundreds of types of bodywork and massage, but the four major categories practiced in the United States are Swedish massage (long, gliding strokes and kneading and stroking), deep-tissue massage (e.g., Rolfing and Hellerwork), pressure-point techniques (e.g., shiatsu and acupressure). and movement integration (e.g., Feldenkrais and Alexander techniques). Nearly every cultural group in the world has a historical tradition of massage therapy.

Training and licensure for massage are variable. Some states require a certifying examination and a statewide license; others regulate massage by municipality. The largest professional national organization of bodyworkers is the American Massage Therapy Association. Membership requires training in an accredited school and hundreds of hours of supervised practice. Most massage schools provide little training in pediatrics, and most massage therapists rarely treat children.

Therapeutic massage can also be provided by physicians, nurses, and physical therapists, as well as by parents and other family members. Massage can be provided alone or in conjunction with guided imagery, music therapy, aromatherapy, or healing touch. Massage is never used as a substitute for conventional care but always as an adjunctive or complementary therapy.

Massage has proved useful for infants, children, and adolescents with diverse health conditions including cancer. [76,77,78,79,80,81,82,83,84] and [85] Massage provides tangible reassurance that the patient is cared for, enhancing self-esteem and a sense of psychological and emotional support. [86] It can reduce symptoms such as nausea, anxiety, and pain; enhance sleep; and promote an overall sense of relaxation and well-being. It can also reduce lymphedema in patients whose lymphatic drainage has been interrupted by surgery or radiation therapy.[87] Massage is also helpful in improving circulation, loosening tight joints, decreasing levels of stress hormones, enhancing endogenous levels of serotonin, and enhancing an overall sense of relaxation and well-being. [80,81]

There are very few contraindications to massage. Common sense precludes the use of vigorous massage over a solid tumor itself, a surgical wound, skin infections, abrasions, or burns. Although time intensive, massage can be provided inexpensively if parents or other family members are trained to do it. Individual adjustments are required for children who are restless or dislike being touched, and those in the midst of typical adolescent conflicts with parents or who have a history of incest or other sexual abuse. Research on the role of massage as an adjunctive therapy for pediatric oncology patients is needed to determine its cost effectiveness under different conditions and to advocate for its inclusion in health insurance benefit programs for persons with cancer. We frequently recommend massage therapy for patients and have hired a part-time massage therapist to provide in-service training to nurses as well as parental instruction on therapeutic massage for pediatric oncology patients.

### *Chiropractic Therapy: Spinal and Cranial Adjustment*

Chiropractic is the leading CAM therapy offered by licensed professionals in the United States. Chiropractors are licensed in all 50 states, and most major insurance carriers cover professional chiropractic care.[88] Children and adolescents typically account for 10% to 20% of all visits to chiropractors. It is estimated that there are approximately 30 million pediatric visits to chiropractors annually, with a 5% to 10% annual growth in the number of visits. [89]

Initial chiropractic visits last an average of 45 minutes, and follow-up visits last 5 to 20 minutes. Nearly all chiropractic schools now offer courses in pediatric care. Although many chiropractors claim to treat otitis media, asthma, allergies, infantile colic, enuresis, and other common childhood health problems, few purport to cure

cancer. Some feel that optimizing spinal alignment helps build resilience in the face of any health condition, resulting in fewer symptoms and a greater likelihood of healing.

There are no randomized, controlled trials demonstrating chiropractic's effectiveness in preventing or treating mild or serious pediatric disorders, and none specifically evaluating its effectiveness as an adjunctive cancer therapy. Acute significant adverse effects from chiropractic adjustments are very rare, however, and the rate of malpractice claims against chiropractors is much lower than that against medical doctors.[90] Research is needed to better understand the use of chiropractic services by oncology patients, their satisfaction with care, and the cost-effectiveness of chiropractic in promoting patients' sense of well-being, quality of life, and symptom management. We have not received any patient requests for referrals to pediatric chiropractors and have not made any such referrals. We have had several requests for referrals to craniosacral therapists and have begun exploring the availability, training, and expertise of such providers in our community.

## Bioenergetic Therapy

### *Acupuncture*

Acupuncture is one component of traditional Chinese medicine.[91] It is based on the theory of a vital energy, chi (qi), that circulates through the body in channels called *meridians*. When the flow of chi is blocked or disrupted, disease occurs; when the flow is balanced, harmonized, and restored, the patient experiences health. The flow of chi can be affected by stimulating specific points along the energy meridians. Approximately 80% of licensed acupuncturists also recommend dietary changes, herbs, and other supplements, and changes in lifestyle, exercise, rest, and relationships.

Despite its exotic nature and its reliance on a different set of assumptions than those of mainstream medicine, acupuncture is frequently endorsed and recommended by physicians and requested by patients, particularly for symptomatic treatment of pain, nausea, and breathlessness.[73,92,93,94,95,96,97,98] and [99] The NIH consensus conference on acupuncture concluded that acupuncture is effective in treating pain and nausea in adults.[100,101,102] and [103] For children, non-needle techniques, such as “sea bands” for nausea, can be used to minimize the risks and fears associated with needles.[104] Among major teaching hospitals with a pediatric pain treatment service, nearly one-third offer acupuncture therapy to treat chronic pain in children.[105] Acupuncture is sought frequently as an adjunctive palliative therapy to minimize nausea, anxiety, breathlessness, fatigue, and a variety pains experienced by cancer patients.[106,107]

Acupuncturists are licensed in more than 20 states, but there is no formal licensure or certification for pediatric acupuncture, and most acupuncturists rarely treat children. Those who do often include non-needle methods of stimulating points—heat, magnets, lasers, and vigorous massage or tapping, particularly for children who are vulnerable to bleeding and infections.[105] Typical visit lengths are 90 minutes for initial and 60 minutes for follow-up visits. Initially, visits are usually recommended two to three times weekly for 2 to 3 weeks, reducing in frequency until no longer needed. Benefits, when achieved, are nearly always notable within the first five treatments.

Despite initial misgivings, most pediatric patients readily accept acupuncture therapy after an age-appropriate introduction to it and an initial treatment.[108] Acupuncture is rarely covered by insurance; the majority of patients pay out of pocket unless a physician acupuncturist provides those services. Side effects from acupuncture treatment, such as infections, broken or retained needles, pneumothorax, and cardiac tamponade, have been reported but are rare.[109,110] Physicians should be aware that acupuncture therapists may also recommend herbs and other therapies, and they should discuss the appropriateness and risks of these therapies specifically with patients, families, and the consulting acupuncturist directly.

Acupuncture therapy is requested by approximately 20% to 30% of patients seen in our consultation service. We have recently hired a part-time pediatric acupuncturist who has 5 years of experience working with the Pain Treatment Service at Childrens' Hospital in Boston and who is well acquainted with the risks of needle insertion in patients with low platelet counts and suppressed immune systems.

### *Healing Touch, Reiki, and Therapeutic Touch*

Healing touch, Reiki, and TT are different kinds of bioenergetic therapy in which the healer transmits a spiritual or invisible healing energy through his or her hands to help patients. They are nonreligious forms of “laying on of hands” healing. These three are the most common types of secular healing techniques in the United States; similar techniques are used in most cultures around the world.

Although these kinds of energy healing techniques seem far-fetched to many physicians, they can be profoundly meaningful to children and families. In our experience, a family's request for information on herbs or other dietary supplements is often a way of testing the waters to determine how their questions or interest in energy healing or spiritual healing will be received by the physician. Because these therapies are rarely used as a replacement for mainstream medicine, and because they are so safe, physicians may wish to consult with nurses, clergy, or others with expertise in these techniques to address families' interests and questions.

Despite its name, TT is typically performed without actually touching the patient. TT was invented in the 1970s by Dolores Krieger, a nursing professor at New York University, and Dora Kunz, a clairvoyant healer. Based on their observations of numerous religious healers, they distilled the process into five secular steps that a healer could use to help patients.[111] These five steps are

1. Having a clear and conscious *intent* to be helpful and heal;
2. Being *centered* in a calm, peaceful state of mind and remembering the patient's innate capacity to experience a sense of peace and well-being;
3. Using the hands to *assess* the patient's energy (typically moving the hands 1 to 3 inches away from the body in a slow downward sweep from the head to the toes);
4. Using the hands to help *restore* the patient's energy to a balanced, harmonious, peaceful state (again, slowing moving the hands a few inches away from the body); and
5. Releasing the patient to complete his or her healing process while the healer returns to his or her own centered, peaceful state of mind.

TT is taught in nursing schools across the United States and in 80 other countries. Formal policies and procedures for performing TT are part of nursing practice in many hospitals, including children's hospitals. There are no national certifying examinations, and no states separately license TT practitioners.

TT is typically used to help reduce symptoms such as anxiety and pain, to enhance rest, and to promote a feeling of general well being. Despite the questionable ability of healers to sense a human energy field, numerous studies in adult, adolescent, and pediatric populations support the use of TT to reduce pain and anxiety and to promote relaxation and a sense of well-being.[112,113,114,115,116] and [117] Fewer studies have evaluated the effectiveness of TT in treating children, but all have reported a sense of relaxation and comfort associated with the technique.[112,118] Practitioners do not promote it as a cure for cancer or as a replacement for standard medical therapies for symptom management, but instead use it to complement mainstream care. Side effects are rare. Costs vary depending on whether the practice is provided as part of routine nursing care or by a parent or other family member.

Reiki is a similar practice that grew out of a Japanese tradition.[119,120] Reiki practitioners are trained by a Reiki master through a workshop and an “empowerment” or “attunement.” Reiki practice relies on a belief in an invisible energy or vital force that may be transmitted from the universe through the healer to patient through intention and placing the hands on particular parts of the patient's body. In some cases, Reiki healers do long distance healing in which the patient is visualized and energy is sent through intention rather than being transmitted by direct physical contact. There are no national certifying examinations, no state licensure, and no studies evaluating its effectiveness in treating children. There are no reported side effects.

In our experience, families who request Reiki therapy or TT are greatly comforted by having it available to their child during hospitalization and during stressful procedures. Many nurses who provide these therapies are also trained in hypnosis, guided imagery, and other techniques that may be helpful in managing stress and anxiety. Providing these therapies directly and teaching them to parents to provide them to children may be one way to communicate the openness, respect, and caring intent of the entire medical team and to enhance patient satisfaction with care. This is a ripe area for research on quality of life issues in pediatric oncology.

### *Prayer*

As oncologists are well aware, every cancer diagnosis presents a potential spiritual crisis for a child and a family. Our experience and numerous studies suggest that

families are eager to discuss the impact of the diagnosis on their spiritual or religious beliefs and often rely on these beliefs as an important coping strategy. [121,122,123 and 124] Most patients and physicians believe that spiritual well-being is an important component of overall health and strongly linked to functional status in cancer patients,[125,126 and 127] yet many physicians feel poorly prepared to address families' questions and concerns in this area.

When used as an adjunctive therapy, intercessory prayer on behalf of patients is low in cost and free of side effects. Scientific studies suggest that it may actually offer tangible health benefits. [128,129,130 and 131] Regardless of its impact on disease or symptom management, it often helps families feel that they are doing everything they can to help the child, reinforces the family's sense of culture and meaning, and promotes a sense of peace and harmony.

Occasionally, physicians are confronted with families who express concerns about medical care and their preference to rely on God or their faith for healing. We have found it helpful in working with such families to acknowledge their beliefs and to ask the family (and the family's spiritual community, if appropriate) to pray for the physicians, nurses, and other health professionals whose mission or calling is to help the child. Making analogies is also helpful—just as we would not expect God to warm our houses without electricity, coal, oil, or wood, we cannot expect God to work without the various tools and persons engaged in the prevention and treatment of disease. Oncologists who feel uncomfortable in these situations may wish to hold a case conference including the family, the child's primary care physician, the institution's pastoral counselors, and the family's own clergy members to assist in addressing these issues before they reach the stage of requiring a court order to override family wishes.

### *Homeopathy*

Homeopathic remedies are commonly used in the United States and even more widely used in Canada, Europe, and India. An estimated 12,000 homeopaths practice in the United States; of these, approximately 50% are lay practitioners, 35% are chiropractors, approximately 10% are physicians, and the rest are naturopaths, nurses, and other health professionals. [132,133]

Homeopathy is a system of medical treatment invented in the 1800s by German physician Samuel Hahnemann. It is based on two principles: (a) the law of similars or “like cures like,” and the (b) the law of dilutions. The law of similars means that a remedy that would cause a symptom in a healthy person is used to treat the same symptom in a sick person. For example a homeopathic remedy made from poison ivy (*Rhus toxicum*) might be used to treat a child suffering from eczema. Although such remedies raise immediate concerns about safety, serious side effects from homeopathic treatment are incredibly rare due to the second principle of homeopathic treatment—the law of dilutions. This law says that the more the remedy is diluted, the more powerful it becomes. Homeopathic practitioners believe that these very dilute remedies contain an energy or information that is used by the patient to heal their symptoms.

Insurance coverage for homeopathic care varies by state, carrier, and the professional status of the practitioner. Homeopathic services provided by physicians and chiropractors are much more likely to be covered than are services provided by nonphysicians. In addition to homeopathic remedies, many practitioners who engage in this therapy also discuss and recommend dietary therapies, dietary supplements, and relaxation techniques. Typically approximately 20% to 30% of a homeopath's patient load are pediatric and adolescent patients. [85]

Homeopathic remedies are available over the counter, through mail order catalogs, and through the Internet without a prescription at relatively low cost. In our experience, families most often select homeopathic remedies, such as Nux Vomica, to treat symptoms such as nausea, or generalized supportive remedies, such as Rescue Remedy, to help minimize the stress and trauma of an acute illness or procedure. We have not encountered families who choose to use homeopathic remedies in place of mainstream cancer care. These remedies are extremely safe and are rarely contraindicated in patients who can tolerate oral preparations. Therefore, we have found it useful to support families in their use of homeopathic remedies; our willingness to support their choice typically results in greater acceptance of and enthusiasm for recommended medical regimens.

## SUMMARY

The use of CAM in pediatric oncology patients is common and increasing. To provide truly comprehensive and compassionate care, oncologists need to be aware of the most common types of therapies, families' reasons and goals in using them, and the resources available within their institutions and through articles, books, and the Internet.

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# PEDIATRIC ONCOLOGY IN COUNTRIES WITH LIMITED RESOURCES

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## INTRODUCTION

Countries with limited resources, also known as emerging nations or (more commonly) developing countries, are defined economically; having an average gross national product (GNP) per capita of $350 (U.S. dollars).[1] There are 125 such countries, and their economic circumstances are highly varied. With the exception of two (Nepal and Yemen Republic), the poorest—those with a GNP per capita of less than or equal to $300—are all in Africa ( Table 55-1). By comparison, the ten richest countries in the world all have a GNP per capita exceeding $25,000 ( Table 55-1). Furthermore, this gap between rich and poor countries continues to grow (Fig. 55-1). By the end of the millennium just closed, the fifth of the world's people who live in the highest income countries had 86% of the world's gross domestic product; the bottom fifth, just 1%.[2] Together, only 12 countries account for 80% of the world's (extremely) poor—defined as those living on less than 1 dollar per day (in 1985 U.S. dollars).[3] These countries are India, China, Brazil, Nigeria, Indonesia, the Philippines, Ethiopia, Pakistan, Mexico, Kenya, Peru, and Nepal; with India and China alone making up 60%. In all of these countries save India, more than half of the population lives in extreme poverty.

| Countries | Gross national product per capita (U.S. dollars) |
|---|---|
| Poor | |
| Burkina Faso | 240 |
| Burundi | 180 |
| Central African Republic | 300 |
| Chad | 230 |
| Congo Democratic Republic | 110 |
| Eritrea | 200 |
| Ethiopia | 100 |
| Madagascar | 250 |
| Malawi | 230 |
| Mali | 250 |
| Mozambique | 210 |
| Nepal | 210 |
| Niger | 190 |
| Nigeria | 300 |
| Rwanda | 230 |
| Sierra Leone | 140 |
| Tanzania | 210 |
| Yemen Republic | 330 |
| Rich | |
| Austria | 26,850 |
| Belgium | 25,380 |
| Denmark | 33,250 |
| Germany | 25,850 |
| Japan | 37,380 |
| Norway | 34,330 |
| Singapore | 30,060 |
| Sweden | 25,620 |
| Switzerland | 40,080 |
| United States | 29,340 |

From Entering the 21st Century: World Development Report 1999/2000. New York: Oxford University Press, 1999, with permission.

**TABLE 55-1. THE RICH AND THE POOR**

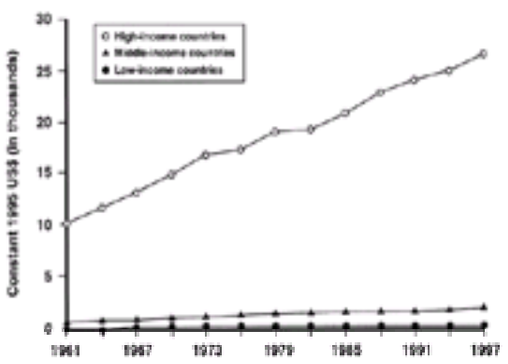


**FIGURE 55-1.** The growing gap between rich and poor countries. (From World Development Indicators. Washington, D.C.: International Bank for Reconstruction and Development/World Bank, 1998, with permission.)

In this chapter we examine cancer in childhood in these underprivileged societies from several perspectives.

1. Epidemiology
2. Notable hurdles
   - Population dynamics
   - Political realities
   - Cultural issues
   - Comorbidities
   - Socioeconomic status
   - Resource restriction and malutilization
   - Elements of cancer control
3. Opportunities for research
4. Strategies for change

This examination may serve to inform the reader of the scope of the challenge in addressing pediatric oncology in countries with limited resources.

## EPIDEMIOLOGY

The magnitude of the burden of childhood cancer worldwide can be estimated only with a rough approximation, for there are very few population-based cancer registries in countries with limited resources.[4] Even where these do exist there is a concern about underascertainment, as exemplified in Namibia[5] and Slovakia.(in

1985 U.S. dollars).[6] However, the apparent age-standardized annual incidence rates overall do not appear to vary markedly around the globe, ranging from 75 to 140 per million.[7] Whether there is a slowly progressive rise, as has been observed in industrialized societies (due mainly to an increase in the incidence of acute lymphoblastic leukemia[8]), is unknown, but the absolute number of incident cases is growing substantially as the population of children expands. The number of new cases annually exceeds 200,000.[9] The proportion of new cases arising in developing countries, currently at about 85%, is rising also and will soon exceed 90%. Unhappily, the proportion of cancer-related deaths in childhood is even higher in these countries and rising faster than the proportion of incident cases. This harsh fact represents a chastening reality; the cure rates enjoyed in the industrialized countries (at present approximately 70% overall and estimated to reach 85% by early in this millennium[10]) are not being experienced by the vast majority of children with cancer.

Regional differences in disease distribution are likely to reflect differences in biology, both in the host and in his or her environment. These have provided important opportunities for study and have lead to enhanced understanding of disease processes. Among the best-known examples are the greater proportions and evidently higher incidences in developing countries of non-Hodgkin's lymphoma (especially Burkitt's lymphoma) and retinoblastoma such that, in some countries, these are the most common forms of cancer in childhood.[7] It is now appreciated that, in large measure, these examples reflect a critical role for infection in pathogenesis (see below). By contrast, the occurrence of Ewing's sarcoma in certain populations—e.g., in blacks and Chinese children—is decidedly uncommon [11] for reasons not yet obvious.

The circumstance of brain tumors is more complex. Apparently striking regional differences in the reported frequencies of primary tumors in the central nervous system seem likely to represent large ascertainment biases, especially of the negative variety. [12] Underdiagnosis may reflect several phenomena, including clinical misassessment and lack of neuroradiological as well as neurosurgical resources. [13,14] In a curious inversion of this situation, the supposed recent increase in the incidence of brain tumors in children in the United States has been attributed to a positive ascertainment bias due to the widespread availability and use of sophisticated neuroradiological techniques.[15] By contrast, there appears to be a real, striking deficit in children of Indian (but not Pakistani) origin living in the United Kingdom.[16]

At a more detailed level, there are regional differences in the proportionality of subgroups of disease that attest also to geographic variation in biology. The most widely studied disease in this regard is acute lymphoblastic leukemia. [17] A higher proportion of cases of T-cell phenotypes is typical of the subgroup distribution in countries with limited resources[17]; even regional variations in the categories within this phenotype have been described—for example, in Egypt [18] and Saudi Arabia.[19] Clues to causal relationships with subgroup distribution are perhaps strongest with acute lymphoblastic leukemia of “common” phenotype, for this appears to have become more frequent (and accompanied by an early age peak) in association with industrialization on retrospective examination over a long period of time in the United Kingdom.[20] A similar but much more abbreviated transition has been described in the Gaza Strip. [21] Again, molecular epidemiological studies of Burkitt's lymphoma have demonstrated different patterns of genetic “lesions” in different geographical areas. [22]

Other regional differences in the biology of individual diseases may likewise shed light on fundamentals of oncogenesis. For example, the relatively common occurrence of Hodgkin's disease in preschool aged children in developing countries (a rare phenomenon in the industrialized world) [23] may reflect the endemicity of and age at first exposure to Epstein-Barr virus infection. In this regard it is of interest that the common occurrence of Hodgkin's disease in very young children was reported more than 20 years ago in Portugal,[24] which was then perhaps the poorest country in Western Europe.

Some such differences remain tantalizing and unexplained. Thus there are the reports of unusual stage distributions of Wilms' tumor in Kenya [25,26] and Zimbabwe,[27] with apparent refractoriness to seemingly adequate conventional therapy. [28] The frequent occurrence of chloromatous manifestations of acute myeloid leukemia reported from Turkey[29] and other countries, especially in Africa, [13] is a further case in point.

Then again, there are a small number of striking regional “oddities,” with unusually frequent cases of skin cancer in Tunisia (on a background of xeroderma pigmentosa)[4] and adrenocortical carcinoma in the neighboring states of São Paulo and Paraná, Brazil, [30] being but two examples. The remarkable prevalence of thyroid cancer, (predominantly of the rare solid papillary type [31]) in children in Belarus and some neighboring locations (interestingly without a comparable increase in acute myeloid leukemia[32]) is all too well explained by the nuclear accident in Chernobyl, Ukraine, in 1986, providing a dramatic lesson in radiation-induced disease.

## NOTABLE HURDLES

### Population Dynamics

As a proportion of the global total, the population of developing countries represented 78% in 1990. [33] However, it is projected that this proportion will rise. Even more striking are the disparities in the proportions of children. Countries with limited resources, in some (the poorest) of which more than 50% of the population is younger than 15 years, accounted for 86% of the world's children in 1990, a figure projected to increase to more than 90% by 2030. [33]

In contrast to some developed (industrialized) countries, in which the birth rate is so low that there is projected to be negative population growth of greater than or equal to 10 million (Table 55-2) until the projected stationary population that is reached after fertility has been at replacement level for many decades, many emerging nations have much higher fertility rates, exceeding six births per woman (Table 55-3). These high fertility rates are accompanied by maternal mortality rates that average almost 1,000 per 100,000 live births (Table 55-3). In striking contrast, the countries with maternal mortality rates less than 10 per 100,000 live births have fertility rates of less than 2, with one exception (Table 55-3).

| Country | Population reduction from 2000 (millions) |
|---|---|
| Germany | 23 |
| Italy | 18 |
| Japan | 30 |
| Russian Federation | 20 |
| Spain | 10 |
| Ukraine | 10 |

*Note:* By contrast, the population of China is projected to increase by 348 million and that of India by 724 million.
From World Development Indicators, 1998. Washington, DC: International Bank for Reconstruction and Development/World Bank, 1998, with permission.

## TABLE 55-2. NEGATIVE POPULATION GROWTH

| Country | Total fertility rate (births per woman, 1997) | Maternal mortality rate (1990–1997)[a] |
|---|---|---|
| Angola | 6.8 | 1,500 |
| Burkina Faso | 6.6 | 930 |
| Burundi | 6.3 | 1,300 |
| Chad | 6.5 | 820 |
| Congo, Democratic Republic | 6.8 | 870 |
| Congo, Republic | 6.1 | 890 |
| Ethiopia | 6.1 | 1,400 |
| Malawi | 6.4 | 620 |
| Mali | 6.6 | 580 |
| Niger | 7.4 | 590 |
| Rwanda | 6.2 | 1,300 |
| Sierra Leone | 6.1 | — |
| Togo | 6.1 | 640 |
| Uganda | 6.6 | 550 |
| Yemen, Republic | 6.4 | 1,400 |
| Australia | 1.8 | 9 |
| Canada | 1.6 | 6 |
| Hong Kong | 1.3 | 7 |
| Czech Republic | 1.2 | 2 |
| Denmark | 1.8 | 9 |
| Israel | 2.7 | 7 |
| Norway | 1.9 | 6 |
| Poland | 1.5 | 5 |
| Slovak Republic | 1.4 | 8 |
| Slovenia | 1.3 | 5 |
| Spain | 1.1 | 7 |
| Sweden | 1.7 | 7 |
| Switzerland | 1.5 | 6 |
| United Kingdom | 1.7 | 9 |

[a] Per 100,000 live births.
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## TABLE 55-3. MATERNAL HEALTH

Despite higher infant mortality rates and shorter life expectancies, these marked differences in fertility rates account in large measure for annual population growth rates for 1978 to 1998 exceeding 4% in some developing countries (curiously concentrated in the Eastern Mediterranean—Djibouti, Jordan, Oman, Qatar, Saudi Arabia, United Arab Emirates, and Yemen),[1] and the inexorable increase in the proportion of children and adults living in countries with limited resources.

However, the impact of these population dynamics is far from uniform within the developing world. For example, in much of the Middle East, health care services are highly developed, readily accessible, and free or heavily subsidized; all in stark contrast to most of sub-Saharan Africa. Consequently, it is no surprise that the management of cancer in childhood is distributed across a very wide spectrum, in both quantitative and qualitative dimensions, in countries with limited resources.

**Political Realities**

As stated so emphatically by the late Dr. James Grant of UNICEF,[34] "Family planning could bring more benefits to more people at less cost than any other technology now available to the human race." In particular, family planning reduces infant mortality by up to 50% if children are spaced by more than 2 years. [35]

Efforts to slow the world's population growth by reducing the birth rate in developing countries is a saga of checkered experience and limited success, however, exemplified by the results of governmental interventions in the two most populous nations on Earth: China and India. In the former, the long-standing official policy of "one family, one child" proved difficult to enforce, especially in rural regions in which it was flouted routinely, and this constraint is now being loosened. The tactics in India have been even less successful,[36] and it has been estimated that the population of India will soon exceed that of China ( Table 55-2). Together, these countries will be home to more than half of the world's population.

Yet successful strategies have been devised. Improving the education of women has resulted in a fall in the birthrate. Indeed, improving the educational level of women results in several health-related gains ( Table 55-4).

| | Percentage contribution of gains in | | |
|---|---|---|---|
| | Income | Educational level of adult women | Generation and use of new knowledge |
| Under-5 mortality rate | 17 | 38 | 45 |
| Female life expectancy at birth | 19 | 32 | 49 |
| Male life expectancy at birth | 20 | 30 | 50 |
| Total fertility rate | 12 | 58 | 29 |

From World Health Report. Geneva: World Health Organization, 1999, with permission.

**TABLE 55-4. SOURCES OF MORTALITY REDUCTION, 1960–1990**

But these laudable gains occur in a context of grossly disparate expenditures on health care—for example, versus military hardware ( Table 55-5)—in developing and industrialized nations and of rampant corruption among public officials leading to a veritable hemorrhaging of capital from countries that can least afford to compromise their limited resources. Conditions such as these foment discord, strife, and outright war. Surely it is salutary to note that as we enter the new millennium there are 120 million antipersonnel mines in 70 countries; one for every 16 children in the world (J. Lemerle, *personal communication*, 1999). These land mines kill up to 10,000 children per year.[38]

| Country | Public percentage of gross domestic product on health | Percentage of central government expenditures on defense |
|---|---|---|
| Burundi | 0.9 | 24.8 |
| Cambodia | 0.7 | — |
| Cameroon | 1.0 | 10.2 |
| Congo, Democratic Republic | 0.2 | 3.7 |
| Gabon | 0.6 | 9.6 |
| Georgia | 0.8 | — |
| Guatemala | 0.9 | 14.2 |
| India | 0.7 | 12.7 |
| Indonesia | 0.7 | 8.9 |
| Myanmar | 0.4 | 12.0 |
| Nigeria | 0.3 | 6.5 |
| Pakistan | 0.8 | 25.3 |
| Paraguay | 1.0 | 7.3 |
| Belgium | 7.0 | 3.5 |
| Croatia | 8.5 | 12.0 |
| Cuba | 7.9 | — |
| Czech Republic | 7.7 | 6.8 |
| France | 8.0 | 6.6 |
| Germany | 8.2 | 5.0 |
| Macedonia, FYR | 7.3 | — |
| Slovenia | 7.4 | 3.4 |
| Switzerland | 7.2 | 6.0 |

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**TABLE 55-5. HEALTH AND MILITARY EXPENDITURES**

Faced with such realities, it is evident that the governments of many developing countries will not be persuaded easily that pediatric oncology is a legitimate priority. In these circumstances, other strategies to effect change must be pursued (see below).

**Cultural Issues**

The very concept of cancer is unknown in many societies; there is no such word in numerous African tribal languages. [13] Consequently, in such cultures, those seeking meaningful discussions with a family about a cancer-related illness in their child encounter an immediate barrier. In other communities, of which there are countless examples in Latin America, explicit communication with the child is forbidden by the family, who themselves may not wish to know the nature of the disease. Again, this poses a challenge to the pediatric oncology team in their efforts to achieve an open dialogue, and it causes serious concerns related to obtaining informed consent and assent. Of course, such dilemmas are encountered in clinical practice in industrialized countries, and there are no simple, broadly applicable solutions.

Preferences for traditional forms of health care intervention are rooted deeply in developing countries and must be respected. For example, the government of India has seen fit to promote traditional healing practices. [39] Indeed, there is much that conventional practitioners can learn from these provider–consumer interactions, including the nature of the interpersonal bond (with its strong elements of trust and compliance) and the biological effects of the interventions prescribed. Similar considerations underlie the widespread use of complementary and alternative medicines in more privileged societies. [40] It is salutary to observe the parallel and contemporaneous practices of traditional and conventional health care in modern hospitals in China and to note that patients are referred easily and commonly from one form of practitioner to the other.

More troublesome is the custom of gender bias, favoring males, in referral for health care, [41] including treatment of cancer,[42] even when gender ratios are adjusted for prevalent and male-dominated lymphomas (Table 55-6). This is but one example of gender inequity, the most extreme forms of which include selective female feticide.[43] Clearly, these practices must be targets for change, just as the male exclusivity with respect to educational opportunities and enfranchisement that exist in some societies is subject to legitimate criticism. Finding ways to effect such equalities is complex and may be founded best on constructive example. The effort is likely to reap reward in the long term, for reduction in illiteracy, which exceeds 90% in adult women in Burkina Faso and Niger, [33] leads to lower fertility rates and improvement in overall health (Table 55-4)—changes that could result in a fall in both the incidence and death rate of cancer in childhood. Formal measurement of changes in gender equity is possible with instruments such as the Gender-related Development Index and the Gender Empowerment Measure ( Table 55-7).

| Country | Childhood population gender ratio (M/F) | Cancer registration gender ratio (M/F) | Lymphoma registration gender ratio (M/F) | Other cancers registration gender ratio (M/F) |
|---|---|---|---|---|
| Bangladesh | 1.07 | 1.57 | 2.88 | 1.88 |
| Egypt | 1.02 | 1.55 | 2.05 | 1.38 |
| Hong Kong | 1.08 | 1.46 | 2.26 | 1.19 |
| India | 1.06 | 1.63 | 2.07 | 1.49 |
| Nigeria | 0.99 | 1.58 | 2.20 | 1.38 |
| Pakistan | – | 2.05 | 3.60 | 1.79 |
| Papua New Guinea | 1.15 | 1.91 | 2.93 | 1.50 |
| Uganda | 0.90 | 1.41 | 1.53 | 1.27 |

From Pearce MS and Parker L. Childhood cancer registrations in the developing world: still more boys than girls. Int J Cancer 2001;91:402, with permission.

**TABLE 55-6. GENDER RATIOS FOR CHILDHOOD CANCER REGISTRATIONS**

| Index | Top five | Bottom five |
|---|---|---|
| Human development index | Canada<br>Norway<br>United States<br>Japan<br>Belgium | Burundi<br>Burkina Faso<br>Ethiopia<br>Niger<br>Sierra Leone |
| Gender-related development index | Canada<br>Norway<br>United States<br>Australia<br>Sweden | Guinea-Bissau<br>Burundi<br>Burkina Faso<br>Ethiopia<br>Niger |
| Gender empowerment measure | Norway<br>Sweden<br>Denmark<br>Canada<br>Germany | Jordan<br>Mauritania<br>Togo<br>Pakistan<br>Niger |

From Human Development Report 1999. United Nations Development Programme. 1999. New York: Oxford University Press, 1999, with permission.

**TABLE 55-7. TOP AND BOTTOM FIVE COUNTRIES IN THE HUMAN DEVELOPMENT INDICES**

Striking success has been achieved in another area that constrains the ability to effect cure in children with cancer in countries with limited resources—namely, the abandonment of therapy. Among the several factors contributing to this problem is the mistaking of remission for cure. Strategies to reduce this phenomenon by an order of magnitude have been reported.[44]

### Comorbidities

The under-5 mortality rate is accepted as the best indicator of the health of a nation's children. [43] As might be expected, in those countries with the highest under-5 mortality rate (exceeding 200 per 1,000 live births)—all of which are in Africa—the life expectancy at birth barely approaches 50 years ( Table 55-8).

| Country | Under-5 mortality rate (per 1,000 live births) | Life expectancy at birth, 1997 (yr) | |
|---|---|---|---|
| | | Males | Females |
| Angola | 209 | 45 | 48 |
| Burundi | 200 | 41 | 44 |
| Malawi | 224 | 43 | 43 |
| Mali | 235 | 49 | 52 |
| Mozambique | 201 | 44 | 47 |
| Rwanda | 209 | 39 | 42 |
| Sierra Leone | 286 | 36 | 39 |

From Entering the 21st Century. World Development Report 1999/2000. New York: Oxford University Press, 1999, with permission.

**TABLE 55-8. QUALITY OF LIFE**

The major killers of children in countries with limited resources remain infections (especially pneumonia, malaria, and measles), malnutrition (including micronutrient deficiencies of iron, iodine, and vitamin A), and diarrheal dehydration. But, these circumstances have undergone considerable change. Respectively, the impacts of immunization programs, nutritional education, and the general availability of inexpensive oral rehydration solutions have been enormous when judged by the numbers of lives saved. These patterns of change are reflected in alterations of regional under-5 mortality rate. [43]

In considerable part as a consequence of these successes, there is an increase in the relative importance of cancer in the spectrum of disease in childhood, in at least some developing countries. For example, in China, cancer is now the most common cause of disease-related death in children of school age, and the same is true in parts of Latin America.

However, there are important interactions between infections, nutritional status, and cancer in early life. The contributions of Epstein-Barr virus to the pathogenesis of Burkitt's lymphoma, Hodgkin's disease, and nasopharyngeal carcinoma are well known, as is the association between the hepatitis B virus (with or without coexposure to aflatoxin) and hepatocellular carcinoma. Perhaps less well known to pediatric oncologists at large is the prevalence of carcinoma of the cervix in adolescent females in developing countries (the highest rate being in Mexico [45]) as a consequence of infection with human papillomavirus (HPV), and the putative association between maternal HPV infection of the genital tract and retinoblastoma in their offspring. Even in Canada, the age-specific prevalence of HPV infection is highest (at 24%) in young women (aged 20 to 24 years).[46] Efforts to develop an HPV vaccine are under way; the impact on public health in general and cancer in young people in developing countries is likely to be important, as has been estimated for the effect of hepatitis B immunization on the death rate from hepatic carcinoma, [47] although hepatitis B vaccine is often unavailable in poor countries in which hepatitis B is prevalent. [47] Likewise, control of HIV infection must be a high priority, especially in African countries in which there has been an increase in the number of cases of Kaposi's sarcoma in children, with a shift from the lymphadenopathic form of the disease to that predominantly involving the skin. [48]

Again, from the standpoint of pathogenesis, there have been notable observations of relationships between maternal diet and cancer in children. A role for *N*-nitroso compounds has been among the most often explored.[49] More recently,there have been multinational investigations of the use of vitamin supplements during pregnancy affording protection from the subsequent development of brain tumors in children, although the results have been inconclusive. [50] The evidence is somewhat stronger that prolonged breast-feeding may reduce the risk in offspring of the subsequent development of Hodgkin's disease. [51] By contrast, this risk may be increased by zinc deficiency.[52]

Provocative observations, related to the use of medicinal plant species of *Euphorbia* in Africa[53] and Asia,[54] point to plausible interactions with Epstein-Barr virus that could promote oncogenesis. Other interactions between infections, nutritional status, and clinical outcomes (morbidity and mortality) are no less important. Thus, endemic bacterial infections combined with poor sanitation obviously endanger the neutropenic child in developing countries. Malnutrition, perhaps as a reflection of low socioeconomic status[55] (see below) and the prevalence of hepatitis B infection in countries with limited resources seriously compromise tolerance of chemotherapy and do threaten survival. Understanding the mechanisms responsible for the effects of malnutrition on drug disposition and pharmacodynamics will be

important contributions to the improvement of prognosis for children with cancer in developing countries. [56] These effects may be compounded by racial differences in drug metabolism resulting from functional enzyme polymorphisms.[57]

**Socioeconomic Status**

Clearly, socioeconomic status is a multielement construct. As a result, definitions vary widely according to the elements included. In Brazil, for example, one pragmatic approach uses an amalgam of household income and quantitated utilization of electricity. [55] This particular construct has demonstrable interrelationships with nutritional status and the prevalence of infectious disease, matters of particular concern in the context of cancer in childhood. Indeed, as defined in the Brazilian scenario, there is a clear association between socioeconomic status and survival, as reported for acute lymphoblastic leukemia. [55]

By what mechanisms does socioeconomic status impact on morbidity and mortality in pediatric oncology? The particularly disadvantaged members of society are less likely to seek conventional health care (being more inclined to consult traditional healers) and have more difficulty accessing medical systems because of limited availability and maldistribution of services, as well as the need to undertake expensive travel. Yet the Convention on the Rights of the Child, adopted more than a decade ago by the United Nations General Assembly, clearly states (Article 21): “State Parties recognize the right of the child to the enjoyment of the highest attainable standard of health and to facilities for the treatment of illness and rehabilitation of health. State Parties shall strive to ensure that no child is deprived of his or her right to access to such health care services.”

Even if access is achieved, affordability is often an issue; in Mumbai, India, as many as one-third of families are forced decline antineoplastic therapy for their children.[58] Chandy[59] has suggested three categories of families on the basis of income, educational status, and motivation to undergo treatment:

1. Illiterate parents working as laborers with a monthly family income of less than U.S. $20, who have little motivation to treat a child with cancer. They constitute 70% of the population in the developing world.
2. Literate parents with average incomes of U.S. $50 to $100 per month, who are well motivated but have considerable difficulty in finding the necessary resources. They constitute 25% of the population in the developing world.
3. Highly educated parents with monthly incomes exceeding U.S. $1,000, who are strongly motivated and possess the resources necessary to support prolonged, intensive treatment. They constitute less than 5% of the population in the developing world.

It is recognized that “income is only a means to human development, not an end”[2] and that a more comprehensive picture of human life, such as the Human Development Index (Table 55-7), is achievable. Indeed, overcoming the cost of treatment may not provide an adequate solution, for subsequent abandonment of conventional care is all too common, as has been described in detail in Central [44] and South America[60] (see below).This is only the most extreme form of reduced compliance with therapeutic protocols, however, because, as was demonstrated in an Australian study, there is a clear relationship between overall compliance and socioeconomic status.[61] Moreover, such problems are not limited to countries listed as having limited resources. There is persuasive evidence from Glasgow, Scotland (one of the poorest cities in Western Europe), that low socioeconomic status is linked to diminished nutritional status and predicts for compromised survival prospects in children with acute lymphoblastic leukemia. [60,62]

Of course, in this context, the issue of physician compliance must not be overlooked. As demonstrated in a Canadian study, [63] this is clearly related inversely to the probability of relapse in children with acute lymphoblastic leukemia.

**Resource Restriction and Malutilization**

Among the most important resources are health care professionals, who are usually in short supply and frequently ill prepared for their roles. There are too few specialized physicians, and they are usually concentrated in big cities, although a large proportion of the population may reside in the countryside and in smaller conurbations—a situation most striking in Africa. For example, in Chad, Eritrea, Gambia, and Malawi there are no more than two physicians per 100,000 people (compared to Israel, which has 459).[2] Access to these often well-trained and capable doctors is limited further by their need to spend the majority of their time in private practice to augment the grossly inadequate incomes provided by the public health care system. Efforts to increase the number of specialized physicians and to expand their availability are well exemplified by the programs of the Indian Academy of Pediatrics. [64]

In many if not most instances, nurses are undereducated and quite simply unable to meet the demands of caring for children with cancer. This major deficit in a cornerstone of clinical practice is widely recognized, and it is now the target of numerous concentrated efforts to bridge the gap by implementing intensive training in local circumstances with the aid of expert nurses from larger centers in industrialized countries. The International Outreach Program of St. Jude Children's Research Hospital has engaged in such activities in Central America, for example.

Not surprisingly, many allied health professions are grievously underrepresented in the clinical teams. Social workers, clinical pharmacists, dietitians, and child life specialists are seldom found. Psychologists, physiotherapists, and others are equally scarce.

A paucity of diagnostic capability, both quantitative and qualitative, regrettably characterizes the lot of those working in countries with limited resources. Within laboratories, there is very little in the way of immunohistochemistry, flow cytometry, ultrastructural analyses, karyotyping, and molecular genetics. In radiology, the availability of computed tomography, magnetic resonance imaging, and the techniques of nuclear medicine is likewise severely restricted, notably in much of Africa. [65] These deficits impose major limitations on diagnostic accuracy, without which decisions on appropriate therapy are jeopardized.

Turning to therapeutic modalities, one has to give immediate attention to the resource-intensive and consumptive aspects of surgical and radiation oncology. The latter is virtually unavailable in much of sub-Saharan Africa, [65,66] whereas sophisticated neurosurgical techniques and limb-salvage procedures are almost unknown in large areas of the developing world.

Perhaps the single most important constraint on progress in pediatric oncology in developing countries is the limited availability of effective chemotherapy. In large measure this is due to costs that are often exorbitant by local standards. The problem is magnified by similar limitations with respect to antibiotics (which are often more expensive than antineoplastic drugs), antiemetics, and other agents used in supportive care. These difficulties may be compounded by cumbersome import regulations, expensive tariffs, and poor inventory control. [13] Attempts to overcome these obstacles, for example, by producing pharmaceuticals locally, do not always meet acceptable standards of quality with respect to safety and efficacy. Despite these impediments, progress can be made even in the most challenging circumstances, as demonstrated by a 60% survival rate in children with stages I–III Burkitt's lymphoma treated with a simplified protocol in Malawi. [67]

In the area of supportive care, there is a particularly difficult challenge regarding blood products. The apparently simple matter of blood donation and collection may be complicated by a wide range of obstacles, from cultural taboos (a donation is viewed as losing part of oneself) to underfunding. Negative attitudes to donation also underlie the racial/ethnic imbalances in the pools of volunteer bone marrow donors in industrialized countries. [68] Strategies to increase the supply of blood products have included the requirement for healthy relatives to provide undirected donations and contracts with commercial companies to provide packed red cells and platelet concentrates in exchange for fractionated plasma. Despite such initiatives, it has been estimated that in India, with more than 1,000 blood banks, only 50% of the need for blood products is met.[69]

Concerns about safety abound and are well founded.[69] This is especially true with respect to the prevalence of hepatitis B and HIV infections in the apparently healthy population, which may be as high as 20% (in China) and 40% (in parts of Africa), respectively. Indeed the HIV-1 seroprevalence in sex workers in Kenya, Uganda, and Zimbabwe exceeds 80%.[3] In India, a considerable fraction of the blood supply is obtained from paid “professional” donors who are mainly men of low socioeconomic status.[70] Testing for hepatitis B and HIV is limited and erratic, and it is estimated that only approximately 50% of transfused blood is properly screened and safe.[70] Faced with these challenges, there is an evident need to be stringent in the use of blood products, [71,72] for example by setting conservative “transfusion triggers” for the correction of euvolemic anemia and the administration of prophylactic platelet concentrates.

All of these shortages must be assessed from the perspective of governmental underinvestments in health care ( Table 55-5). Unfortunately, there appears to be little prospect of relief from foreign aid, which is in global decline ( Table 55-9). These realities have placed severe demands on nongovernmental organizations such as the African Medical and Research Foundation and have taxed the ingenuity (as well as the patience and tolerance) of health care providers in countries with limited

resources, especially in Africa.[65]

| Country | Annual average percentage change in volume, 1991–1992 to 1993–1994 |
|---|---|
| Australia | +1.7 |
| Austria | +1.5 |
| Belgium | −2.6 |
| Canada | −8.1 |
| Denmark | +3.5 |
| Finland | −14.2 |
| France | −2.2 |
| Germany | −2.2 |
| Ireland | +18.8 |
| Italy | −9.4 |
| Japan | −3.6 |
| Luxembourg | +9.2 |
| Netherlands | +0.1 |
| New Zealand | +0.5 |
| Norway | −0.2 |
| Portugal | −0.2 |
| Spain | +2.1 |
| Sweden | −2.2 |
| Switzerland | +0.3 |
| United Kingdom | +1.1 |
| United States | −8.0 |
| Total | −3.2 |

From World Development Indicators. Washington, D.C.: International Bank for Reconstruction and Development/World Bank, 1996, with permission.

**TABLE 55-9. NET OFFICIAL DEVELOPMENT ASSISTANCE-AID FLOW**

The amalgam of resource restrictions combine to limit access to care that, in a dozen countries (all but two of which are in Africa), is afforded to less than half of the population (Table 55-10).

| Country | Proportion of the population with access (%) |
|---|---|
| Angola | 24 |
| Benin | 42 |
| Cameroon | 15 |
| Central African Republic | 13 |
| Chad | 26 |
| Ghana | 25 |
| Guinea | 45 |
| Haiti | 45 |
| Indonesia | 43 |
| Mozambique | 30 |
| Niger | 30 |
| Senegal | 40 |

From World Development Indicators, Washington, D.C.: International Bank for Reconstruction and Development/World Bank, 1998, with permission.

**TABLE 55-10. ACCESS TO HEALTH CARE**

### Elements of Cancer Control

There are few opportunities to effect prevention of cancers that occur during childhood and adolescence, but the prospects are not entirely bleak. Educational programs aimed at reducing sun exposure, avoiding unprotected sex, and changing some regional habits (such as the chewing of betel nuts and smokeless tobacco) could impact the prevalence, respectively, of malignant melanoma, HIV-associated Kaposi's sarcoma, and oral cancer. More indirectly, counseling parents (especially during pregnancy) with respect to dietary exposures (see above) may have an additional benefit. At least as important, global efforts to encourage young people not to smoke predictably will have a major impact on the incidences of tobacco-related cancers (and other illnesses) in adult life—surely an enormously worthwhile goal—although recent efforts attest to the refractoriness of the problem.[73]

As to the element of screening, numerous challenges are presented. In the circumstance of familial retinoblastoma, the need for early and repeated examination of the eyes is obvious. Whether such a practice would have a role in parts of the world in which the sporadic disease is most common remains to be tested. It is unlikely that screening for neuroblastoma will be useful in countries with limited resources because the incidence of this disease in general is lower than that in industrialized societies in which screening programs have not affected an appreciable reduction in morbidity or mortality.[74] By contrast, there is good evidence for the role of routine cervical smear examinations in reducing the burden of illness associated with carcinoma of the cervix, a disease that does occur among girls in developing countries.[45] The even easier practices of breast and testicular self-examination can be taught readily to adolescents[75] and could have measurable benefit. It remains to be determined whether the training of primary health care workers in developing countries in the Integrated Management of Childhood Illness, as advocated by the World Health Organization,[76] will result in the earlier detection of more cases of cancer in children.

Treatment is the most important element of cancer control in childhood. Although it is manifestly important to promote adherence to fundamental oncological principles in surgical practice (as with needle biopsies, sampling of lymph nodes, and obtaining clear lines of resection), surgeons are ever more commonly facing the challenges of operating after neoadjuvant therapy. Because children with solid tumors often are referred initially to surgeons (who may be the only practitioners of oncology in some parts of the world), fostering modern surgical contributions to the care of children with cancer can be difficult, demanding a combination of patience, persistence, and continuing education. With radiotherapy, the challenges are materially different; relating more to issues of availability, equipment costs (including maintenance), quality control, and appropriate use.[13,66] Conversely, delivering cost-effective chemotherapy requires that close attention be paid to the therapeutic index, the often precarious balance between efficacy and toxicity. Walking this line can pose particular hazards in the context of comorbidities (see the section Comorbidities). Detailed knowledge of local circumstances can allow the elaboration of appropriate treatment strategies, as exemplified by the Burkitt's lymphoma project in Malawi.[67]

Acceptable effectiveness may require considerable treatment intensity, especially if high-dose chemotherapy and multi-modality approaches are used. There is no role for half-hearted therapy, which is associated predictably with limited effectiveness,[63] but treatment intensity comes at the price of toxicity. As a result, the importance of supportive care cannot be overstated.[77] The essential components of infection control, pain management, antiemesis, blood product supply, and nutritional support must be in place. In countries with limited resources, provision of supportive care can be especially demanding, often generating imaginative and innovative solutions which should be (and sometimes are) incorporated in the therapeutic armamentarium of less resource-restricted centers. Among these may be cited the safe and effective delivery of high-dose methotrexate with “rescue” on an ambulatory basis[78]; outpatient management of fever and neutropenia with oral antibiotics[79] (now emulated cost effectively in the United States[80]); and the administration of supplemented candy bars as nutritional support.[81] Even allogeneic bone marrow transplantation has been accomplished without hospital admission in the developing country setting.[82]

Palliative care outside of the hospital environment may seem like an insuperable problem in countries with limited resources, especially as it remains a major challenge even in the most privileged circumstances.[83] Yet it is in these situations that the great majority of deaths from cancer in childhood occur. When there is sufficient will to address the issue, surprising success may ensue, as in the case of the hospice established more than 10 years ago in Kenya by the late Edward Kasili (Dr. W. Macharia, *personal communication*, 2000). The related matter of bereavement counseling is equally ripe for development, requiring culturally sensitive approaches to ensure acceptability.[84] Such considerations are embodied in Children's Hospice International (Veronica Feeg, *personal communication*, 2000).

For those children who survive malignant disease, long-term follow-up and assessment of quality of life become focal points in health care. Follow-up is especially difficult in developing countries, but again persistence can be rewarded, as exemplified by studies of former patients with Burkitt's lymphoma in Uganda.[22] Likewise, efforts to measure the health-related quality of life of survivors should not be viewed as a superfluous luxury. Such measurements can be made with ease and have been accomplished in countries with limited resources with surprising results (Table 55-11). These achievements allow the calculation of disability-adjusted life years, judged by the World Health Organization to be important measures of the comprehensive health status of individuals and populations.[37]

| | Respondents | |
|---|---|---|
| | Parents | Physicians |
| Latin America[a] (SR, HR) | 0.93 | 0.98 |
| Canada | | |
| SR | — | 0.96 |
| HR | — | 0.90 |
| Controls[b] | 0.95 | — |

HR, high-risk disease; SR, standard-risk disease.
*Note*: Utility scores range from 0 (dead) to 1 (perfectly healthy).
[a]A consortium of centers in Colombia, Cuba, Honduras, and Uruguay.
[b]Children in the general Canadian population.
From Barr R, Gonzalez A, Longchong M. Health-related quality of life (HRQL) in survivors of cancer in childhood in Latin America. Int J Oncol 2001;19:413.

**TABLE 55-11. HEALTH-RELATED QUALITY OF LIFE (MEAN UTILITY SCORES) OF SURVIVORS OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDHOOD**

## OPPORTUNITIES FOR RESEARCH

Contrary to a prevalent supposition that countries with limited resources cannot afford to support and conduct research, the case for essential national health research has been made persuasively.[85,86] This cogent argument is stimulated in part by the evident need to find locally appropriate, cost-effective solutions to local problems and opportunities. Among these is the challenge of caring for children with cancer, determined by the World Bank to be a cost-effective undertaking in developing countries.[87] Particularly expensive procedures, such as bone marrow transplantation, are proper targets for economic evaluation. [88]

In this setting, there are particular prospects worthy of attention that should attract the investment of more privileged parties, notably in sophisticated technology. The epidemiological evidence of widely disparate distributions of disease has prompted interest in genetic predisposition and molecular oncogenesis, especially with respect to viral etiology. Coupled with the markedly different circumstances of rural and urban populations that provide fertile ground for investigations of potential environmental pathogenesis, such studies should shed new light on the biology of cancer in childhood around the world. Moreover, there is the added value of technology transfer in such “North-South” cooperative ventures. A striking example is provided by the recent discovery of a novel, consistent, germline mutation in children with adrenocortical tumors in Brazil. [89]

The obvious survival advantage offered to children with cancer by participation in therapeutic trials [90] while receiving care in specialized centers [10] provides a sound rationale for fostering clinical research in pediatric oncology in countries with limited resources. At the same time, we must be devoting attention to the ethical responsibilities of investigators. [91] Participation in such endeavors, which include the essentiality of good data management, enhances the discipline of health care delivery and establishes a higher standard of practice. These goals are promoted by the formation of cooperative groups. Successful examples of such enterprises exist in Latin America, within individual countries [such El Grupo Argentino de Tratamiento de la Leucemia Aguda in Argentina (GATLA), the Wilms' tumor consortium in Brazil, and the National Chilean Pediatric Oncology Group (Programa Nacional Infantil Drogas Antineoplasicas; PINDA)], as well as multinationally [as with Grupo Latino Americano de Tratamiento de Hemopatias Malignas (GLATHEM) in South America and Asociación de Hemato-Oncologia Pediatrica Centro-Americana (AHOPCA) in Central America]. These entities have built a solid track record, producing outcomes rivaling those reported from equivalent clinical trials in Europe and North America.[92,93,94] and [95] Again, there is added value—in this instance it is the building of knowledge and experience.

## STRATEGIES FOR CHANGE

The remarkable progress in pediatric oncology enjoyed in industrialized societies has come at increasing cost. This escalating financial burden, on families and on health care systems,[96] highlights and amplifies the inadequate resources available in developing countries for the care of children with cancer. Such a widening gap strains the abilities of governments to meet the need and prompts consideration of “alliances of stakeholders” to address and remedy the deficits. [97] Associations of parents and volunteers with physicians and other health professionals provides a realistic framework for progress in developing countries.

Sharing experience and resources is the basis of the successful “twinning” programs between well-established pediatric oncology centers in developing countries and aspiring institutions in countries with limited resources, as espoused in the Montevideo Document. [98] These programs must be real partnerships of equals—addressing objectives approved bilaterally—to be mutually rewarding. Such is the case between a consortium of centers in German-speaking countries and numerous institutions in Eastern Europe; between the National Cancer Institute in the United States and a group of partners in India; between various centers in Italy and their one-to-one linkages in Latin America (one of the successful outcomes from the Monza International School for Pediatric Hematology-Oncology [99]); and withthe International Outreach Program of St. Jude Children's Research Hospital and its network, including institutions in the Middle East. One of the most important platforms on which these programs have been built is the exchange of personnel with a focus on training. The long-term impact of these educational opportunities is enormous. In large measure, however, these have involved only physicians, laboratory scientists, and nurses so far. There is a major need to extend opportunities of this kind to data managers, clinical pharmacists, and other member groups of multidisciplinary pediatric oncology health care teams. Success in such ventures should lead to the establishment of pediatric oncology units,[9] functioning as local centers of excellence. It is even possible to envisage such centers engaging in shared care with nonspecialist health care professionals (such as general pediatricians) who work closer to the home of the child and family—a model proposed for the large and populous country of India. [64]

The prospects for sustainable success would be enhanced measurably if funds were provided to free pediatric oncologists from the need to generate the majority of their income in private practice. This would enable them to devote more of their time to exercising the leadership on which past accomplishments have been based and future progress depends so heavily.

However, even these twinning programs have restricted scope. A broader, more inclusive alliance of stakeholders is needed. [97] In particular, the value of involving parents has become obvious. Local and national organizations have formed an international confederation—the International Confederation of Childhood Cancer Parent Organizations. This organization is affiliated with the International Society for Pediatric Oncology with which it holds contemporaneous annual meetings. The potential role for International Confederation of Childhood Cancer Parent Organizations in advocating for increased resource allocation, to address the imminent needs of children with cancer and their families, is enormous. As an example, a local organization (Grupo de Apoio ao Adolescente e à Criança com Câncer, or GRAACC) in São Paulo, Brazil, raised sufficient funds (entirely from nongovernmental sources) to build and operate an 11-floor institute devoted exclusively to pediatric oncology.[100] Parents may make valuable contributions also in more “hands-on” ways, as in the reduction of abandonment of therapy reported from Nicaragua.[101] It is likely also that they could play important roles in the continuing debate about ethical standards in the conduct of clinical research in developing countries.[91] In many countries with limited resources, however, parents do not alone have the skills to fulfill such roles; there, the establishment of community-based support groups can prove to be invaluable.

On a wider front, it will be important to harness the expertise of appropriate organizations (including the International Association of Cancer Registries) to increase the number of population-based cancer registries in developing countries [102] using the international classification of childhood cancer [103] and to forge liaisons with international pharmaceutical companies to provide drugs more affordably and to support therapeutic trials. As governments are interested increasingly in economic evaluation of health care technologies, including clinical interventions, so, too, there are opportunities to perform cost analyses and match these with measurements of health status and health-related quality of life to better inform health care planning and policy development. [104] There are even emerging accounts of measures of the quality of care provided by hospitals in developing countries. [105] The recently established International Network for Cancer Treatment and Research is poised to play a leadership role in meeting many of these challenges.

Bringing all of these elements together will be facilitated by the tools of electronic communication (on-line journals, telemedicine, and the UNICEF Web sites, among others). Providing these amenities and the training to use them is sure to foster the continuing development of pediatric oncology in countries with limited resources.

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# 56

# PREVENTING CANCER IN ADULTHOOD: ADVICE FOR THE PEDIATRICIAN

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## INTRODUCTION

The pediatric oncologist's primary responsibility is treating and curing a child with cancer with the intent of increasing the quantity and quality of life. General pediatricians, other primary care physicians, and health care providers alike are also responsible for ensuring that their pediatric patients attain the highest quantity and quality of life. Each of these providers is concerned with reducing the risk of new malignancies throughout their patients' lives, in childhood years as well as into the adult years. Pediatricians have a unique opportunity to teach and promote cancer prevention practices. This chapter describes precepts of cancer prevention and control aimed at children for the purpose of preventing cancer in adulthood.

Pediatric malignancies represent an extremely small proportion of all incident cancers in the United States—less than 1%. Although the incidence of pediatric cancer is low, its significance is high. Knowledge about the fundamental genetic basis of cancer has been derived largely from work in pediatric oncology. Cancer is the second leading cause of death for adults in the United States, as it is for children. Numerically, the occurrence of adult cancer has a dramatic impact on the general population; only deaths due to cardiovascular disease outnumber cancer deaths. Public health concern about the adult cancer burden is related not only to incidence and mortality but also to societal economic costs.

Some determinants of adult cancer can be identified during childhood. For inherited cancer susceptibility factors such as germline mutations of tumor suppressor genes or oncogenes, cancer predisposition can be determined at birth or potentially *in utero*. Exposure to exogenous substances identified as risk factors for adult cancer often begins during childhood. Evolving knowledge about the interplay between exogenous exposures and inherited genetic susceptibility can serve as the basis for the development of cancer prevention strategies.

The goal of this chapter is to review known determinants of adult malignancies and to discuss possible preventive interventions that might be applied throughout childhood and adolescence to reduce the incidence, cancer-related morbidity, and mortality of adult cancer. Children represent a captive audience for health education interventions; they may have more “teachable” moments, and small changes in lifestyle adopted during childhood can have dramatic effects on future cancer risk. For children with known genetic predisposition syndromes, introduction of early detection and surveillance screening practices, often performed many years before incident disease, can reduce the treatment-related morbidity and economic cost of cancer treatment. The pediatrician is uniquely qualified to instill cancer prevention practices that will derive public health benefits over many decades.

## CHARACTERISTICS OF ADULT CANCER

Although nearly one-third of the U.S. population is younger than 20 years, only approximately 12,000 new cases of cancer will occur annually in this group. In contrast, more than 184,000 incident female breast cancers were expected to occur in the United States in the year 2000.

Anatomic site of occurrence characterizes adult malignancies, as most tumors are derived from epithelial tissue. Most childhood cancers originate in the neuroectoderm- and mesoderm-derived tissues and are characterized by histologic features. Table 56-1 shows the estimated new cancer cases and deaths for the year 2000. For women, the most common adult malignancies are cancer of the breast, lung, colon, and rectum. For men, cancer of the prostate, lung, colon, and rectum occur most frequently. The highest numbers of cancer deaths occur with the lung, colorectal, and breast cancers.

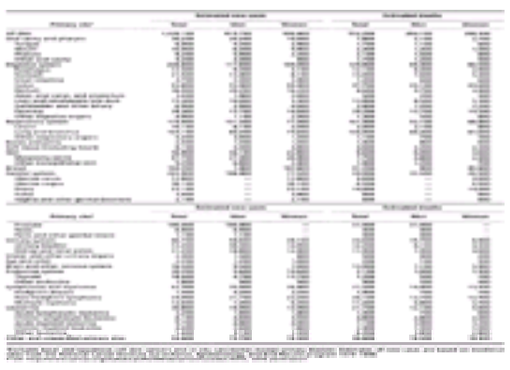

**TABLE 56-1. ESTIMATED NEW CANCER CASES AND DEATHS IN 2000 FOR THE UNITED STATES**

## METHODS OF CANCER PREVENTION

Cancer risk is related to the interaction between genetic factors and environmental exposures. An unusual clustering of cancer within families provided the first evidence that cancer was a genetic disease. Although genetic susceptibility is important, only a small proportion of the incidence is attributable to inherited genetic predisposition factors. Cumulative exposure to tobacco smoke, occupational exposure to asbestos and chemicals, exposure to carcinogenic air pollutants, and harmful dietary exposures are usually required along with acquired susceptibility to cross a biological threshold at which tumor formation and ultimate metastasis can occur. Although host genetic susceptibility factors such as germline mutations can contribute to that process, they are usually insufficient to serve as single etiologic causes. For example, in the two-hit model of cancer causation for retinoblastoma, the first “hit” is a germline mutation that is present in every cell at birth. An additional mutation, usually from an exogenous exposure, is required for development of this malignancy.

Effective cancer prevention strategies take advantage of eliminating exposure to known cancer-causing exogenous substances, slowing the rate of transition of accumulated cellular genetic changes, or increasing exposure to protective exogenous agents. In addition, interventions that assist in detecting cancer at the earliest stages of development, although not related to the onset of disease, can reduce the morbidity and mortality of cancer.

Many adult cancers are amenable to prevention. Initiating cancer prevention practices during childhood and adolescence may offer the most effective means of reducing cancer risk in adulthood. These practices include interventions designed to reduce exposure to harmful agents and increase exposure to protective substances and implementation of screening and early detection practices in childhood or even during the prenatal period, with benefits accruing to individuals over many decades of life. Genetic counseling may even provide a means to prevent adult cancer preconceptually.

*Cancer control and prevention* is defined as a research science aimed at reducing cancer incidence, morbidity, and mortality.[1] This research builds on laboratory and clinical investigation and emphasizes early detection and screening, clinical intervention, and health education aimed at minority, traditionally underserved, and high-risk populations. Cancer control priorities are established on the basis of incidence and mortality rates in the population, availability of methods to modify exposure to known risk factors, and availability of effective treatments. Figure 56-1 depicts the cancer prevention model with transition through stages of disease. Blocking the onset of disease is referred to as *primary prevention*. Blocking the advancement of cancer after its onset but before definitive diagnosis is referred to as *secondary prevention*. Interventions aimed at enhancing long-term outcome, including therapies to eradicate newly diagnosed malignancy as well as efforts to reduce treatment-related morbidity or improve health-related quality of life, are referred to as *tertiary prevention*. The focus of this chapter is on primary and secondary prevention methods to reduce the adult cancer burden.

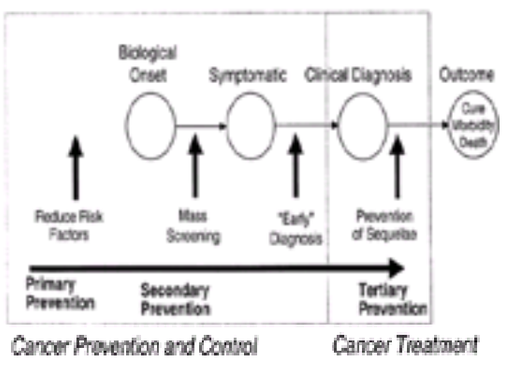


**FIGURE 56-1.** A schematic diagram of the cancer prevention model.

### Primary Prevention

The most efficacious and cost-effective prevention strategies are to reduce exposure to harmful substances or increase exposure to beneficial agents, leading to a reduction in the incidence of cancer. Intervention at this level is primary prevention. Primary prevention usually provides the most efficient means of lowering cancer-specific mortality and morbidity as well as reducing the consequent economic and psychosocial impact of disease. As a prerequisite, a causal association between one or more putative risk factors and disease must be demonstrated. Effective methods to reduce exposure must be available. For pediatric malignancies, causal factors accounting for the majority of disease incidence have not been identified. In fact, most of these cancers may result from spontaneous (“background”) rather than induced somatic mutations; therefore, primary prevention is not feasible. In contrast, for many common adult malignancies such as head and neck cancer, lung cancer, and esophageal cancer, most of the incidence can be attributed to tobacco use, an environmental agent in this case, making primary prevention feasible.

### Secondary Prevention

Secondary prevention is the detection of cancer in early stages of development. Implicit is the requirement that early diagnosis results in reduced mortality and morbidity. Less invasive therapy is required, treatment is less costly, and the incidence of treatment-related sequelae is lower. The benefits of a screening and early detection program must be evaluated in relation to the associated psychosocial and ethically negative impact that results from assignment of “high-risk” status. The effect of misclassification (false-negatives and false-positives) must also be accounted for. Screening and early detection may offer the only practical means of positively influencing cancer outcome when primary prevention is not possible or practical. In this new millennium, large investments are being made in the identification of agents that will delay or prevent the transition from early to late disease, thus increasing the utility of secondary prevention.

### Cancer Prevention Strategies

An organized cancer prevention and control research program requires the multidisciplinary interaction of primary care physicians, oncologists, epidemiologists, molecular biologists, geneticists, toxicologists, and health educators, as well as public policy specialists and health care economists. These individuals must work in tandem to develop strategies to identify determinants of cancer risk, reduce exposure to risk factors, encourage protective behaviors, and develop early detection interventions.

## GENETIC BASIS FOR CANCER

Cancer is the result of a multistage process in which loss of control of cellular growth and differentiation occurs. Table 56-2 illustrates a hypothetical model of tumor development and progression for colorectal cancer.[2] The process starts with an inherited germline mutation or acquired mutation in the *APC* gene. This single mutation leads to the formation of dysplastic aberrant cryptic foci and early adenomas in the colon, usually following mutation, inactivation, or loss of the second copy of the gene. Next, an acquired *KRAS* gene mutation frequently occurs in one of the benign tumors, allowing one of the cells to outgrow its sister cells and form a larger intermediate, dysplastic adenoma. Within this population of cells, further mutations or losses occur, including those in the *TP53* tumor suppressor gene. Acquired *TP53* mutations lead to loss of apoptosis. When a cell acquires a sufficient number of mutations, metastatic potential is achieved. Thus, the entire process may require four or more sequential mutations. The microenvironment of an organ such as the colon can further affect cellular growth and proliferation of a malignant clone of cells.

| Tissue state | Genetic mutation |
|---|---|
| Normal epithelium | |
| ↓ | *APC* |
| Dysplastic aberrant cryptic foci | |
| ↓ | |
| Early adenoma | |
| ↓ | *KRAS* |
| Intermediate adenoma | |
| ↓ | DCC/DPC4/JV18 |
| Late adenoma | |
| ↓ | *TP53* |
| Carcinoma | |
| ↓ | |
| Metastasis | |

**TABLE 56-2. PATHOGENESIS OF COLORECTAL CANCER THROUGH ACCUMULATION OF SEQUENTIAL MUTATIONS**

The generalized model of carcinogenesis includes several theoretical steps. Initiation is marked by the occurrence of the first cellular genetic abnormality, consisting of a mutation that can be transmitted to progeny cells. Promotion leads to the development of benign tumors or focal proliferative lesions. Progression marks the conversion of these tumors or focal proliferative lesions into malignant tumors. Although initiation, promotion, and progression are well characterized in experimentally induced cancers, they are usually not clinically observable. For adult malignancies, the often subtle nature of carcinogenesis makes it difficult to differentiate between discrete transition states, such as changes in normal cells through the stages of proliferation, dysplasia, and *in situ* carcinoma. At a cellular level, cancer can be thought of as a genetic disease of somatic tissue. Blocking or slowing the rate of transition forms the fundamental basis for cancer control and prevention.

## Cancer Family Syndromes

### *Syndromes Affecting Children*

Historically, familial cancer syndromes as well as observed cytogenetic changes, such as the Philadelphia chromosome in chronic myelogenous leukemia, provided important clues linking genetics with cancer. Retinoblastoma provided the first genetic model connecting hereditary and nonhereditary forms of the same cancer. The clinical observation that children presenting with bilateral disease at young ages suggested an autosomal dominant pattern of inheritance. [3] Cytogenetically, for 5% to 10% of these cases, chromosomal band 13q14 was completely or partially deleted in all cells of the body. [4] Friend and colleagues [5] cloned the *RB1* gene in 1986, establishing the mechanism of how tumor suppressor genes work. Mutations or deletions at tumor suppressor gene sites result in total reduction or production of dysfunctional proteins that normally regulate cellular growth and proliferation. In this case, the two alleles at the *RB1* locus produce the RB protein (pRb); pRb inhibits uncontrolled cellular proliferation. Children with a germline mutation have only one of the two normal alleles that produce normal pRb. All retinoblast cells inherit this condition and are therefore more susceptible to development of cancer caused by a new somatic mutation; the cancers are two-hit lesions. Such mutations would knock out the remaining normal allele, leading to the development of retinoblastoma. Another well-characterized tumor suppressor gene is *TP53*, which codes for the protein p53. Mutation or loss of pRb leads to an increased birth rate of cancer cells; *TP53* mutations lead to a loss of normal p53, affecting apoptosis and resulting in a decreased death rate for cancer cells. Germline *TP53* mutations are associated with the Li-Fraumeni cancer family syndrome. [6] These individuals are predisposed to a wide spectrum of pediatric and adult malignancies. Most familial cancer syndromes are associated with a relatively limited number of malignancies, and none of the known germline mutations predispose to all cancers.

### *Syndromes Affecting Adults*

Cancers that appear in adulthood have also been associated with germline mutations. Although they account for a relatively small proportion of breast cancers in the general population, [7] mutations in *BRCA1* and *BRCA2* are strongly associated with familial breast cancer. [8] Similarly, although less than 10% of ovarian cancers are familial, the majority of familial cancers involve *BRCA1* or *BRCA2* mutations. [9] *APC* is a tumor suppressor gene associated with colorectal cancer. von Hippel-Lindau disease is a cancer family syndrome associated with a number of adult malignancies, including renal cell carcinoma. The *VHL* gene acts as a tumor suppressor gene. If germline mutations such as those listed above can be detected in childhood, the potential exists for early detection and possible prevention of adult cancer.

What can be done with knowledge that a child possesses a germline mutation associated with adult cancer? For a familial breast cancer example, girls identified with *BRCA1* or *BRCA2* germline mutations can receive mammography screening more frequently and at an earlier age than the recommended guidelines. Given that hormone levels are likely to have an etiologic role in breast cancer, the use of antiestrogens, modification in the timing of pregnancy, as well as the use of prophylactic mastectomy or oophorectomy in extremely high-risk individuals might all be considered as possible preventive measures. Prophylactic thyroidectomy might be considered for children with familial multiple endocrine neoplasia types IIA and IIB. Annual colonoscopy starting early in adulthood or prophylactic colectomy might be considered for children with hereditary nonpolyposis colorectal cancer syndrome or familial adenomatous polyposis (FAP) syndrome, although the use of cyclooxygenase inhibitors shows promise for the latter disorder.

## Polymorphic Genes

Genetic susceptibility polymorphisms affect cancer risk. There are a number of classes of these polymorphisms, an important one being those that code for xenobiotic metabolizing enzymes such as cytochrome P-450, glutathione S-transferase, and N-acetyltransferase. Polymorphisms of metabolizing enzymes are associated with detoxification of exogenous contaminants or metabolism of relatively inactive contaminants into active carcinogens. Another class includes polymorphic genes that code for enzymes regulating DNA repair, including the genes *hMLH1* and *hMSH2*. From a population perspective, cancer susceptibility polymorphisms are likely to account for the observed interindividual variation in response to identical levels of carcinogen exposure. As pharmacogenomic technology advances, we are likely to develop more accurate predictive models of cancer risk and thus target more aggressive interventions toward extremely high-risk individuals.

## Characteristics of Adult Cancer Compared with Childhood Cancer

The wide variation of incidence patterns geographically and the fact that migrants ultimately show different incidence rates in their new countries reflect the multifactorial etiology of cancer and point to environmental factors in the origin of many adult cancers. Although familial cancer syndromes are implicated as causes in a defined subset of the population, their overall impact on cancer incidence is small. However, well-studied familial cancer cases reveal that the tumors often display the same changes found in the nonhereditary forms. A consequence is that preventive measures that are efficacious for high-risk hereditary cases should be helpful for nonhereditary cases. At present, we know far more about potentially modifiable risk factors for adult malignancies than we do about childhood malignancies.

Adult malignancies may require a greater number of sequence-dependent genetic “hits” (i.e., mutations) than those required for pediatric malignancies. Colorectal cancer probably requires four or more hits. [10] Given the greater number of required hits, adult malignancies are more likely to develop later in life and may be more dependent on other potentially modifiable risk factors; therefore, there are more opportunities to block or delay the process of adult cancer development.

# RISK FACTORS FOR ADULT CANCER

In addition to spontaneous mutations, exogenous factors represent important determinants of cancer risk. Because exogenous exposures are usually modifiable, most of the common adult cancers are theoretically preventable. From a multifactorial perspective, the challenges are to identify these factors and their interactions with other factors and to develop interventions designed to modify exposure or decrease host susceptibility. Ultimately, it is the impact on cancer incidence, mortality, and morbidity that establish the utility of these interventions. This process should lead to the formation of policies and regulations that minimize public exposure to harmful substances or increase exposure to protective substances.

Endogenous factors (e.g., genetic, hormonal, and immunologic) also play an important etiologic role. However, control over exposure to these factors is often impossible to manipulate. For example, it is currently impossible to repair germline mutations in the *APC* locus to reduce cancer risk. Such control may eventually be possible with gene therapy; however, the only current means of lowering risk for individuals with inherited cancer susceptibility is to control exogenous exposure or use secondary prevention to improve cancer outcome.

The response to environmental exposures and host factors is not limited to the first stage of cancer development—initiation. The response can extend to the later stages of promotion and progression. Multiple pathways can exist for a single agent, and multiple agents can interact with themselves and with host characteristics. Carcinogenesis of breast, colon, and prostate are thought to involve alternate pathways for oncogenesis. The pathogenesis of cancers of the upper airway and digestive tract, such as oral, pharyngeal, esophageal, and lung cancer, may be simpler because tobacco use and alcohol consumption are known to be more than sufficient to cause the majority of these malignancies. Other host factors, such as constitutional defective DNA repair mechanisms, tumor suppressor gene mutations, and immunodeficiency, may explain the variation in incidence patterns among individuals exposed to the same levels of tobacco and alcohol.

## Host Characteristics

Host characteristics play a pivotal etiologic role in human cancer. Given the multifactorial nature of oncogenesis, host factors work in tandem with exogenous exposures to increase cancer risk. The major categories of cancer-associated host characteristics are reviewed in sections that follow.

### *Genetic Factors*

There have been a number of germline mutations that increase the risk of familial cancers. In the population, rare genetic disorders such as retinoblastoma, ataxia-telangiectasia (AT), neurofibromatosis, von Hippel-Lindau disease, multiple endocrine neoplasia, and Li-Fraumeni syndrome collectively account for little of the overall cancer incidence. However, they clearly demonstrate that cancer is a genetic disease. Even genetic syndromes that can be identified in childhood portend a high risk of developing new malignancies in adulthood.

The mutation for AT that causes this rare autosomal recessive disorder and fatal neurologic disease has been found on chromosome 11q22-23. [11] The AT gene is a genetic cancer predisposition factor for heterozygote carriers. These nonafflicted individuals express a nearly fourfold increased risk of breast cancer [12] due to increased sensitivity to ionizing radiation and defective cell cycle control. [13] The estimated prevalence of AT carriers in the United States is approximately 2.5 million, or approximately 1% of the population. Therefore, 4% of breast cancer cases could carry the AT mutation. DNA testing could be used to identify these carriers. Given their increased sensitivity to radiation, these individuals may be cautioned to strictly avoid all elective radiation exposure, such as routine medical or dental radiographic imaging.

### *Hormonal Factors*

Endogenous hormones play a role in the etiology of breast cancer. [14,15] Estradiol and progesterone levels and their age-dependent exposure pattern of use are associated with breast cancer. Early age at menarche, nulliparity, late age at menopause, and late age at first full-term pregnancy are risk factors. [16]

### *Immunologic Factors*

Inherited or acquired immunodeficiencies are risk factors for malignancy. [17,18] Immunodeficiency syndromes that are associated with cancer include AT, severe combined immunodeficiency syndrome, acquired immunodeficiency syndrome, Wiskott-Aldrich syndrome, and Bloom syndrome. Autoimmune syndromes have also been implicated as risk factors. Acquired immunodeficiencies due to infection with human immunodeficiency virus (HIV), thymectomy, chemotherapy, radiation exposure, or multimodal therapies such as bone marrow transplantation can also increase cancer risk.

Other medical conditions, such as inflammatory bowel disease, thought to be an autoimmune-mediated process, or infection with viruses such as HIV that can impair T-cell–mediated immune surveillance, are associated with increased cancer risk. Although HIV is not directly involved in cellular transformation, it may impair normal immune function[19] and may enhance the oncogenic potential of viral coinfections, such as human herpes virus 8 or Epstein-Barr virus (EBV), which are associated with B-cell lymphomas and leukemias[20,21] and [22] and smooth muscle tumors.[23,24]

## Exogenous Exposures

Host characteristics alone account for a very small proportion of cancer incidence. However, exposure to chemical, physical, and biologic factors is known to play a pivotal causal role for the most common adult malignancies. The activity of a single exogenous risk factor has to be considered relative to the exposure level, exposure duration, biologic response, and interactions with other factors. Chemical exposures (reviewed by Yuspa and Shields [25]) serve as initiators, promoters, or factors that aid progression. Chemical carcinogens can be genotoxic. These are highly reactive agents that can directly bind to DNA to form DNA adducts, in turn leading to base mispairing, small deletions, missense or nonsense mutations, chromosomal breaks, or large deletions. Nongenotoxic chemicals rarely interact directly with cellular DNA. They can serve as modifiers of effect for genotoxic agents or act independently as endocrine or immune disruptors.

Chemical exposures occur in occupational as well as residential settings. Children can be exposed to high levels of pesticides if they live near agricultural areas, and they can be exposed to industrial contaminants if they live or play near polluted areas. Tobacco smoke contains more than 40 components that are known carcinogens. Foods may contain artificial or naturally occurring substances that are harmful (e.g., aflatoxins). Potentially harmful physical exposures include ionizing radiation (e.g., x-rays, nuclear fallout, and residential radon [26]), ultraviolet radiation from sun exposure or artificial ultraviolet light sources, [27] electromagnetic fields, and asbestos.

Historically, the etiologic effects of high-dose ionizing radiation are well known from the atomic bombing of the cities Hiroshima and Nagasaki, [28,29] the widespread use of fluoroscopy used to fit shoes, radiographic imaging used to monitor tuberculosis, [30] and irradiation for treatment of diseases. [31,32] The effects of very-low-dose x-ray exposure are not precisely known. The latency between exposure to ionizing radiation and cancer suggests that this is an initiating event, leaving open the contributory carcinogenic effects of other factors.

The single most important preventable known risk factor for adult cancer is tobacco use, including cigarette, cigar, and pipe tobacco as well as smokeless tobacco products. One-third of cancer deaths are likely related to tobacco use. Tobacco use is strongly associated with cancers of the upper airway and digestive system and moderately associated with cancer of the bladder, uterine cervix, pancreas, and kidney. In addition to being a primary cause of cancer, tobacco use is a major risk factor for coronary artery disease, cerebrovascular disease, and respiratory disease.

Infectious agents account for a high proportion of malignancies in the developing world. Examples include Burkitt's lymphoma in equatorial Africa, nasopharyngeal carcinoma in China, and gastric cancer in Chile. Infectious agents can exert a direct carcinogenic effect, or their effects can be indirect, such as induction of chronic cellular proliferation leading to an increase in the number of spontaneous accumulated mutations. A number of viral agents have been associated with malignancy, including EBV, human papillomaviruses (HPVs), hepatitis B, hepatitis C, retroviruses, such as the human T-cell lymphoproliferative viruses (HTLV-I and HTLV-II) and HIV-1 and HIV-2. The bacterium *Helicobacter pylori* is associated with gastric carcinoma and low-grade gastric mucosa–associated lymphoid tissue lymphoma. [33]

Host factors and coinfection with other organisms can amplify the effect of an infectious agent. For example, early age of infection with EBV and coinfection with malaria (*Plasmodium falciparum*) predispose the individual to the endemic form of Burkitt's lymphoma. Acquired immunodeficiency syndrome–associated malignancies represent the interaction of HIV-induced immunodeficiency and EBV-related lymphoproliferation. [23] Nasopharyngeal carcinoma is related to EBV infection, genetic susceptibility in geographically defined populations (such as in China), and dietary exposure to *N*-nitroso compounds.[34]

Other malignancy associations with infectious agents include: HPV (types 16, 18, 33, and 39) with cervical and anorectal cancers, hepatitis B and C with hepatocellular carcinoma, and adult T-cell malignancies with HTLV-I. *Mycobacterium tuberculosis* has been identified as a possible risk factor for bronchogenic carcinoma, and schistosomiasis has been identified as a risk factor for bladder cancer. [35]

# PREVENTION AND CONTROL STRATEGIES FOR ADULT CANCERS

## Primary Prevention

### *Dietary Factors*

International ecologic data have correlated diets high in fat with increased risk of breast cancer. [36] Studies of Japanese migrants to Hawaii have demonstrated increased breast cancer risk with adoption of a more Western lifestyle. [37] However, data from large prospective cohort studies have been more controversial, providing little direct evidence linking total or saturated fat intake with increased breast cancer risk. [38] Diets higher in fruits and vegetables have been studied for prostate cancer. These results have failed to show a clear association between dietary modification and a consequent reduction in cancer incidence. [39] Consumption of cruciferous vegetables,[40] lycopene, b-carotene, lutein, zeaxanthin, and polyphenolic antioxidants (in green tea) has been examined. The relationship between these agents and cancer has been inconsistent perhaps, because of the methodologic complexities of administering food intake questionnaires, the relatively small size of many of the study populations, or the limited follow-up duration. Novel approaches have included the combination of dietary interventions with assessment of polymorphisms of metabolizing enzymes. A differential protective effect of flavonoids against lung cancer was demonstrated in individuals with specific cytochrome P-450 CYP1A1 genotypes.[41]

Dietary recommendations must be based on solid evidence of a protective effect. At present, this evidence is weak. Although increasing exposure to food substances found to be protective *in vitro* or *in vivo* seems straightforward, these experimental results have not held up in clinical intervention trials. The scientific knowledge base necessary to justify dietary recommendations and policies is insufficient at present and is confounded by the widespread and growing use of nutritional supplements

in the general population.

***Tobacco Use***

Tobacco use represents the single most important modifiable risk factor for cancer. The prevalence of smoking for adults in the United States has declined since the 1980s; however, the rate of decline has slowed in recent years. After first declining, smoking prevalence rates in young people flattened and have shown a steady increase in recent years. For both adults and youth, tobacco use is related to gender, education, and minority status. For the period 1990 to 1996 overall cancer incidence and mortality declined, as did the incidence and mortality for lung cancer. The decline has been greatest in men, with a continuing increase in lung cancer mortality for women. This can be explained by the decreasing rate of smoking in men and an increasing rate in women, and by the fact that men began smoking earlier in the twentieth century.

Antismoking research seeks to accurately characterize smoking patterns in the population, understand the etiology of smoking, develop effective strategies to prevent new individuals from beginning to smoke, and develop smoking cessation interventions. Success in quitting smoking is related to the magnitude of addiction or dependence, presence of other smokers in the smoker's social environment, a history or proneness to depression, and the use of coping mechanisms. [42]

Recent litigation against the tobacco companies has resulted in multibillion-dollar settlements for many of the states. Portions of these funds were spent on mass media campaigns, including community-based antismoking interventions for children and adolescents. Multimillion-dollar health education and mass communication programs were initiated. In Florida, the Florida Pilot Program on Tobacco Control has achieved success in reducing tobacco use behavior in middle school and high school students.[43] Using an organized mass media approach targeted at middle and high school students, current cigarette use has dropped, the prevalence of “never-use” increased, and the percentage of adolescents with committed “nonsmoking attitudes” has increased. Workplace smoking restrictions have also been successful in reducing the rate of new adolescent smoking,[44] as have compliance checks for retailers selling tobacco products. [45]

Physicians who provide care for children have an important responsibility to counsel their patients to avoid tobacco use. Preventing tobacco use is far more effective than achieving cessation of an already established cigarette smoking habit. Strategies to prevent tobacco use include practice-based, community-based, and school-based interventions, and policy and advertising regulation.

Physicians can take an active role in smoking cessation efforts and can prescribe a range of medications to aid in the process. [46] Increased regulation of tobacco sales, increases in taxes, and new litigation are likely to continue the decrease in smoking prevalence. On June 27, 2000, President Clinton issued an Executive Memorandum directing all federal agencies to encourage their employees to stop or prevent tobacco use. [47] Other policies, such as those designed to increase social stigmatization and bans on smoking in public places, will further these efforts.

***Infectious Disease Intervention***

The prevention or control of certain infections is an important means to prevent cancer. For example, long-term antibiotic treatment may reduce the risk of gastric cancer for individuals with *H. pylori* infections.[33] Widespread vaccination for hepatitis B will lower incidence rates for hepatocellular carcinoma. Malarial control along with improved hygiene may decrease the incidence of endemic Burkitt's lymphoma by shifting EBV infection to older ages. Teaching safe sexual practices, such as the use of condoms, may cut down the rate of HPV transmission, the major cause of cervical cancer. These safe sex practices also favorably reduce HIV transmission rates.

***Reducing Exposure to Sunlight***

Exposure to sunlight and sunburn, especially during early childhood, are the principal causes of melanoma; the rates of this malignancy have been declining, except in older men.[48] Study of migrants from the United Kingdom to Australia suggests that exposure at young ages and duration of stay in Australia affected melanoma risk.[49] Techniques such as skin self-examination,[50] health education interventions aimed at increasing sun-protection behavior. [51] and even chemoprevention[52] have the potential to reduce melanoma risk.

***Chemoprevention***

Chemoprevention is the application of agents to inhibit, delay, or reverse carcinogenesis. These agents include many classes of compounds such as antioxidants (both naturally occurring as well as synthetic compounds, such as vitamin E, vitamin C, selenium, isoflavinoids, and retinoids), antiinflammatories [nonsteroidal antiinflammatory drugs, cyclooxygenases (cyclooxygenase-2 inhibitors)], and synthetic hormones that are antiestrogenic, such as tamoxifen. Potentially effective chemoprevention agents must be evaluated in the same manner as other therapeutic agents. Testing would include phase I through phase III trials as well as postmarketing evaluations. Tools such as high-throughput DNA microarray analysis, cell culture, and the development of transgenic and gene knockout mice are aiding the discovery of new chemotherapeutic as well as chemopreventive agents. Targeting of individuals in chemoprevention trials should be based on the relative risk of that person developing cancer. Individuals with genetic cancer syndromes may be singled out for chemopreventive therapy. Surrogate end points are often used to demonstrate the activity of these chemopreventive agents over a relatively short follow-up duration.

**Secondary Prevention**

Secondary prevention is the identification of cancer in earlier stages of its natural history. Early detection and screening can be accomplished by means of simple self-examination (e.g., breast self-examination and testicular self-examination), use of sensitive advanced technologies such as radioimaging, image-guided biopsy, or detection of tumor markers (e.g., serum prostate-specific antigen). Biomarkers are biochemical or cellular compounds that can identify cancer susceptibility or the effects of tumorigenesis. They can serve as sensitive tools to indicate the presence of precancerous lesions or asymptomatic malignant tumors. Biomarkers for susceptibility can be used to identify high-risk subsets for invasive tests. They can also be used to indicate the disappearance or regression of early lesions, including those that occur spontaneously and those induced from early therapeutic responses. Biomarkers that detect cellular changes within tumors, such as biomarkers for angiogenesis, can mark tumor progression. Late steps of tumorigenesis are often marked by detectable *RAS* or *TP53* mutations.

Prerequisites for secondary prevention include the availability of an accurate method to detect cancer or a premalignant state and evidence that early therapeutic intervention will result in better outcomes. Early detection and screening methods must first be shown to be valid (i.e., highly sensitive and specific) and cost-effective. Costs must be considered in terms of economic, physical (e.g., morbidity and mortality of invasive tests), and psychosocial impact. Few screening tests are available to detect childhood cancer. The Quebec Neuroblastoma Screening Project evaluated the use of a mass screening test for infant neuroblastoma. Although this screening program showed a shift toward identifying earlier stage disease at the time of diagnosis, there was no significant impact on overall mortality. [53] In contrast, screening has been shown to lower mortality for breast and colorectal cancer.

FAP screening is an example of how early detection might be used in a pediatric setting. Screening for mutations in the *APC* locus would allow children to be identified as high risk for the development of colorectal cancer later in life. Although the onset of cancer can occur in the second decade of life, cancers typically do not develop until the third or fourth decades of life. The baseline level of suspicion about the presence of an *APC* mutation is not high unless there is a remarkable family history of colorectal cancer. Detection of *APC* mutations in childhood could positively influence cancer prevention practices such as introduction of dietary modification, chemoprevention, and increased surveillance with annual colonoscopy and polypectomy starting in adolescence. For extremely high-risk cases, prophylactic colectomy may be a viable medical option.

## HEALTH POLICY ISSUES RELATED TO PREVENTION DURING CHILDHOOD

A rational cancer prevention program must include ready access to health care services, public health education aimed at promoting healthy lifestyles including tobacco use avoidance, dietary improvement, and the possible use of chemoprevention agents. Coupled with improved secondary prevention methods, a combination of these interventions can lead to significant decreases in cancer incidence and mortality.

For children, genetic testing for adult cancer susceptibility is uncommon. Although the level of suspicion for cancer predisposition may be higher for families that have experienced an unusual number of cancers affecting both children and adults, such as *TP53* germline mutation–associated childhood gliomas and soft tissue sarcomas and adult small cell lung cancers and breast cancers, the utility of genetic screening during childhood is less obvious for predisposition syndromes that

affect only adults, such as FAP-related colorectal cancer. Theoretically, identifying these adult predisposition syndromes at young ages should confer advantages over their discovery in adulthood.

From an economic perspective, identifying high-risk populations based on genetic testing can improve the efficacy of surveillance measures. Expensive and invasive early detection screening tests can be reserved for those individuals determined to be at highest risk for cancer, thus stretching limited economic resources.

On a more subtle level, as we uncover new germline mutations, learn more about the role of polymorphisms of genes that affect cancer susceptibility, and begin to develop the bioinformatics infrastructure needed to link DNA microarray data with clinical data, it may become possible to accurately predict individual cancer risk over a person's lifetime. Individuals can be identified that are most likely to benefit from avoidance of environmental, occupational, or lifestyle-determined exposures such as agrochemicals, low-dose radiation, tobacco, and sexually transmitted infectious organisms.

Reduction in the prevalence of high-risk behaviors is more efficient if these behaviors can be prevented rather than abated. Adoption of lifelong health-promoting behaviors, including prevention of tobacco use, adherence to dietary guidelines, reductions in infection with oncogenic viruses, and adherence to recommended screening and early detection guidelines should be started early in life. Molecular epidemiologic screening for children can identify the subset with very high-risk features that warrant increased surveillance. Genetic counseling may be offered to families to reduce the probability of transmitting cancer susceptibility to the next generation.[54,55 and 56]

As screening for cancer susceptibility becomes more widespread, the physician can help identify individuals most likely to benefit from primary prevention (e.g., chemoprevention and prophylactic surgery) or secondary prevention (e.g., screening only high-risk individuals that result in higher test yields). However, current technologies do not allow for prediction of an individual's eventual disease probability with sufficient precision to cast aside all concerns about the negative effects of genetic testing. How best to communicate the results of genetic tests remains controversial; more behavioral research is needed to establish rational guidelines for informing children and their families about test results. Even these well-conceived judgments can change as new interventions are developed.

The physician's office and school-, community-, and population-based settings are venues to communicate cancer prevention messages. Given the general emphasis on prevention, the family practitioner, general internist, and pediatrician can play pivotal roles in encouraging health-promoting behaviors for their patients. The implementation of governmental policies that encourage cancer preventive interventions for children, that reduce access barriers for prevention services, and that strengthen safeguards against the public's exposure to potentially harmful substances should be part of the nation's cancer plan.

Multifactorial etiology characterizes adult and pediatric malignancies. Constitutional germline mutations and DNA polymorphisms, acquired genetic mutations, and exposure to exogenous substances in the environment or through diet may operate concomitantly to cause a malignancy. Because our knowledge about risk factors is more extensive, and given the longer latency between exposure and disease onset, adult malignancies are potentially more amenable to prevention.

The refinement of methods for high-throughput and low-cost DNA testing, along with other advances in pharmacogenomics and bioinformatics, will hasten the deployment of wide-scale screening for cancer susceptibility with high levels of precision. Thus, for children with a pharmacogenomic profile that places them at exceptionally high risk for carcinogenic damage from polycyclic aromatic hydrocarbons combined with genetically predetermined defective DNA repair capacity, we may be able to construct risk profiles to ensure these high-risk children avoid high-exposure occupations.

Research is ongoing to identify markers for enhanced endocrine or immune disruption in response to toxins such as chlorinated hydrocarbons. Polymorphic differences that affect the metabolism of toxins and drugs suggest an increased role for ecogenetics.

Although mass screening for gene-related cancer susceptibility may be on the horizon, the societal implications have not been fully thought through. For example, for children with a history of FAP, there is burgeoning evidence to suggest that genetic testing has minimal psychological impact on children or their parents when offered in an organized setting with readily available counseling.[57] However, the long-term impact is unknown. Issues such as future insurability, family planning, and the cost and burden of frequent screenings need to be quantitated. As the technology for genetic screening becomes more readily available, we will be faced with issues such as social discrimination and stigmatization that must be balanced against the benefits of genetic testing. All of the costs—monetary, emotional, and societal—must be weighed against the medical benefits. For individuals identified as being at high risk, decision-analytic methods might be used to guide the choice of interventions. Preference-based measures taken from a patient's perspective might assist in choosing strategies with alternate surveillance schedules as well as assisting with selection of radical interventions such as prophylactic colectomy, oophorectomy, or mastectomy.

Health policies that favor application of preventive interventions during childhood can lead to significant reductions in cancer burden in adult years. Genetic testing can provide a means to improve the cost-effectiveness of surveillance in individuals with known or suspected familial cancer susceptibility. Testing for colorectal cancer with conventional methods such as colonoscopy and sigmoidoscopy can be improved when combined with genetic testing results.[58]

## RECOMMENDATIONS

Prevention of adult cancer should begin during childhood and adolescence. The sequential and often lengthy process of carcinogenesis for adult malignancies suggests that there are opportunities to block transitions at multiple points. Doing so should provide the most cost-effective means of preventing cancer. Exposures during childhood are known to be important for certain adult cancers such as breast cancer (i.e., hormone levels in late adolescence) and melanoma (i.e., repeated severe sunburns during childhood). Simple interventions, such as changing the formulation of oral contraceptives (e.g., reduced steroid dose by use of a gonadotropin-releasing hormone agonist with very-low-dose estrogens and intermittent progestogens), using sunblock and adhering to safe-sunning practices, encouraging sexual abstinence or the use of condoms to reduce the transmission of infectious oncogenic agents, and reducing exposure to tobacco, can significantly lower cancer risk.

Ensuring ready access to routine health care is important. Certain population subsets defined by characteristics such as socioeconomic status, race, ethnicity, culture, or geography cannot or do not avail themselves of routine medical care. Access to routine care is also affected by age. Similar to the problems related to providing continuity of care for childhood cancer survivors, “well-care” for the general pediatric population greatly diminishes throughout adolescence and may be nonexistent for young adults. The responsibility for providing routine medical care falls somewhere between the pediatrician and internist. Physicians need to ensure that adolescents receive routine preventive care so that cancer prevention behaviors can be initiated and maintained as early as possible.

Pediatricians and generalists who care for children and adolescents should make cancer prevention a priority in their practice. Health education efforts have been shown to lower smoking prevalence. Targeted efforts should be made to reach out to traditionally underserved pediatric populations. Continuity of medical care must be provided from late adolescence through early adulthood to ensure that children avail themselves of all available cancer prevention services. As gene therapy is developed, genetic predisposition syndromes may be correctable. For now, detection of increased cancer susceptibility during childhood can lead to more effective early detection strategies. Pediatricians have a critical role to play in educating their patients to choose healthy lifestyle behaviors that will ultimately have a dramatic impact on cancer incidence and mortality.

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# RESOURCES FOR CHILDREN WITH CANCER, THEIR FAMILIES, AND PHYSICIANS

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## INTRODUCTION

This chapter includes information on many of the organizations around the world that provide support, diagnostic services, and treatment to children with cancer. Additionally, information is included on many of the professional societies and organizations that function to promote professional collaboration in pediatric oncology and related fields. E-mail and Web addresses have been included, when possible, to facilitate communication with these organizations.

The chapter is organized into three sections: The first section lists community and support resources, indexed by keywords so that organizations providing particular services are more easily identified. The organizations are listed by country in alphabetical order. Resources specific to the United States government also are provided in this section. In the second section is a list of organizations that provide diagnostic and treatment services. Organizations are listed by country in alphabetical order. Finally, section three lists organizations designed to support professionals working in pediatric oncology and related fields. These organizations are listed in alphabetical order by title of the organization.

The information in this chapter is subject to change. Readers should contact the individual organizations for the most up-to-date information.